Optimization of methodology for the simultaneous speciation of inorganic As, Sb and Se in fluid samples by sector-field ICP-MS coupled to HPLC

Dissertation

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Contents

Abstract

Metal speciation provides information useful in the study of toxicity, bioavailability, adsorption, and redox behavior of element species. Based on inductively coupled plasma mass spectrometry (ICP-MS) coupled to high performance liquid chromatography (HPLC), in this project, a systematic investigation was made regarding chromatographic methods for the simultaneous speciation of arsenic (As), antimony (Sb) and selenium (Se) redox couples, and preservation strategies of these species. Finally, the developed method was applied to the analysis of hydrothermal water samples, with the purpose of studying As and Sb inorganic species distribution in hydrothermal systems.

In the first study, a new method was developed for the simultaneous speciation analysis of inorganic As(III, V), Sb (III, V) and Se(IV, VI) in fluid samples by sector field-ICP-MS coupled with HPLC. Hamilton PRX-X100 anion-exchange column with EDTA (pH of 4.7) and 3% methanol as mobile phase was used for the separation of these species. The overall analysis time was within 11minutes for all six desired species. A thorough validation concerning stability of retention time, linearity and spike recovery was carried out. Low detection limits of these species, 0.02 μg L^{-1} for As(III), 0.06 μg L^{-1} for As(V), 0.2 μg L^{-1} for Sb(III), 0.02 μg L^{-1} for Sb(V), 0.2 μg L^{-1} for Se(VI) and 0.4 μg L^{-1} for Se(IV), make it possible for simultaneous study of competitive adsorption, redox behavior of these species.

In the second study, preservation method and stability of As, Sb and Se redox couples were investigated in Fe- and Mn- rich water samples (groundwater, river water and lake water). As potential preservation strategies EDTA alone and EDTA combined with either HCl, HNO_3, formic acid or acetic acid were studied and compared to unpreserved samples. The results showed that addition of EDTA combined with acidification to a pH of 3 successfully preserved all three redox couples for at least 11 weeks stored at 4 °C in the dark. EDTA alone (pH = 6) failed to preserve As and Sb species, especially for Sb(III) which was eventually completely oxidized in all samples. On the other hand, in the unpreserved samples, As, Sb and Se redox species showed different adsorption behaviors. As(III), Sb(III), Se(IV)) and As(V) were strongly adsorbed by Fe-

(oxy)hydroxide and possibly Mn-(oxy)hydroxide. While Sb(V) and Se(VI) were not adsorbed in most cases.

In the third study, the developed speciation method was used for the analysis of hydrothermal waters from Bali and Java, Indonesia. The results showed that the distribution of As and Sb species were closely correlated to Cl^-, HCO_3^- and SO_4^{2-}. Generally, in HCO_3-type hydrothermal waters As(V) seemed the dominant species. In Cl-type samples, it is more complicated. Since extremely high concentration of Cl might be originated from either magma degassing (HCl gas) or seawater feeding, thus other oxidation processes may be involved in As species distribution. Our primary speciation results indicated that when the hydrothermal waters were affected by seawater feeding, As(V) was the main existing form, probably due to microbial activity. In SO_4-type hydrothermal waters, As distribution is variable, either As(III) or As(V) could be the dominant species. In addition, an unknown As species was detected in 5 of the 18 samples, particularly in 2 samples this unknown species was even the main existing form for As, indicating that microbial activity was involved. For Sb species, Sb(V) was generally the main species in the analyzed samples.

Kurzfassung

Metallspeziation liefert Informationen, die sehr wichtig für die Untersuchung der Toxizität, der Bioverfügbarkeit, der Adsorption und des Redoxverhaltens von Elementspezies sind. Basierend auf der Methode der Massenspektrometrie mittels induktiv gekoppleten Plasma, das mit einer Hochleistungsflüssigchromatographie (HPLC)-Apparatur verbundenen war, wurden im Rahmen dieses Projektes systematische Untersuchungen zu chromatographischen Methoden für die simultane Speziation von Arsen (As)-, Antimon (Sb)- und Selen (Se)-Redoxpaaren sowie von Konservierungsstrategien dieser Spezies durchgeführt. Anschließlich wurde die entwickelte Methode für die Analyse von hydrothermalen Wasserproben angewandt.

Im Rahmen der ersten Studie wurde eine neue Methode für die simultane Speziationsanalyse von anorganischem As(III, V), Sb(III, V) und Se(IV, VI) in Fluidproben mittels einer mit einer HPLC gekoppelt an ein Sektorfeld-ICP-MS entwickelt. Für die Trennung dieser Spezies wurde dabei eine Hamilton PRX-X100 Anionenaustauschersäule mit EDTA (pH 4.7) und Methanol (3%) als mobile Phase verwendet. Die Gesamtanalysenzeit für alle sechs gewünschten Spezies lag innerhalb von 11 Minuten. Darüber hinaus wurde eine gründliche Validation hinsichtlich der Stabilität der Retentionszeit, der Linearität und der Spike-Wiederfindung durchgeführt. Die niedrigen Nachweisgrenzen dieser Spezies (0.02 µg L^{-1} für As(III), 0.06 µg L^{-1} für As(V), 0.2 µg L^{-1} für Sb(III), 0.02 µg L^{-1} für Sb(V), 0.2 µg L^{-1} für Se(VI) und 0.4 µg L^{-1} für Se(IV)) ermöglichten die simultane Untersuchung konkurrierender Adsorptions- und Redoxverhalten dieser Spezies.

In der zweiten Studie wurden einerseits Konservierungsmethoden und andererseits die Stabilität von As-, Sb- und Se-Redoxpaaren in Fe- und Mn-reichen Wasserproben (Grund-, Fluss- und Seewasser) untersucht. Als potentielle Konservierungsstrategien wurden sowohl EDTA, als auch EDTA in Kombination mit entweder HCl, HNO_3, Ameisensäure oder Essigsäure untersucht und die Ergebnisse mit denen nicht-konservierter Proben verglichen. Es zeigte sich, dass sich alle drei Redoxpaare durch die Zugabe von EDTA und die Ansäuerung auf pH 3 erfolgreich für mindestens 11 Wochen dunkel gelagert bei 4°C konservieren ließen. EDTA alleine (pH 6) war nicht in

der Lage, As- und Sb-Spezies zu konservieren. Dies gilt insbesondere für Sb(III), das in allen Proben letztendlich vollständig oxidiert wurde. In den nicht-konservierten Proben zeigten die As-, Sb- und Se-Redoxspezies dagegen unterschiedliche Adsorptionsverhalten. As(III), Sb(III), Se(IV) und As(V) adsorbierten stark an Fe- und möglicherweise auch Mn-(oxi)hydroxiden, während Sb(V) und Se(VI) in den meisten Fällen nicht adsorbierte.

In der dritten Studie wurde die entwickelte Speziationsmethode für die Analyse von hydrothermalen Wässern aus Bali und Java (Indonesien) verwendet. Die Ergebnisse zeigten, dass die Verteilung von As- und Sb-Spezies sehr eng mit den Gehalten an Cl^-, HCO_3^- und SO_4^{2-} korreliert. Im Allgemeinen schien As(V) die dominierende Spezies in hydrothermalen Wässern des HCO_3-Typs zu sein. In Proben des Cl-Typs ist es komplizierter. Da extrem hohe Cloridkonzentrationen entweder von HCl ausgasendem Magma oder Kontakt mit Meerwasser herrühren können, mögen andere Oxidationsprozesse bei der Verteilung von As-Spezies beteiligt sein. Unsere primären Speziationsergebnisse zeigten, dass As(V) die vorherrschende Spezies darstellte, wenn hydrothermale Wässer durch Meerwasserspeisung beeinflusst sind, was möglicherweise auf mikrobielle Aktivität zurpückzuführen ist. In hydrothermalen Wässern des SO_4-Typs ist die Verteilung des As variable, sowohl As(III), als auch As(V) können die dominierende Spezies sein. Zusätzlich wurde eine unbekannte As-Spezies in fünf der 18 Proben gefunden, wobei diese in zwei Proben sogar die wichtigste Form darstellte, was auf eine Beteiligung mikrobieller Aktivität hindeutet. Im Falle des Sb war Sb(V) in der Regel die Hauptspezies in den analysierten Proben.

1. Introduction

1.1 As, Sb and Se in aqueous environment

1.1.1 As

Arsenic (As), a metalloid occurs naturally, being the 20th most abundant element in the terrestrial crust (Gulledge and O'Connor, 1973). Arsenic and its compounds are mobile in natural environment. Rock-weathering converts As sulfides to As trioxides, which subsequently enter into the aquatic environment by dissolving in rain, rivers, or groundwater. Arsenic has only one stable isotope, ^{75}As. It can exist in the -III, -I, 0, III, and V oxidation states. Arsenic is highly toxic and leads to a wide range of health problems in humans. If entering the food chain, As accumulates in animal bodies in the form of organic species. Arsenic has become increasingly important because of natural water contamination as well as human activities, *e.g.* industrial waste and drainage problem. Numerous studies have shown that excessive intake of As from drinking water can lead to chronic poisoning and various types of cancers, *e.g.* skin, lungs, bladder and kidney (Smedley and Kinniburgh, 1993). Arsenic has been classified as a group I human carcinogen by the International Agency for Research on Cancer due to the increased cancer risk. The maximum permissible levels of As in drinking water have been reduced in many countries. The United States Environmental Protection Agency (USEPA, 2006) set the maximum contaminant level in drinking water at 10.0 μg L^{-1}, the same as the guidelines of the World Health Organization (WHO). Australia has a drinking water limit for arsenic of 7.0 μg L^{-1} (NHMRC, 2004). The American Natural Resources Defense Council (NRDC) even recommended an As level of 3.0 μg L^{-1} (NRDC-report, 2000).

Arsenic concentrations in natural environment can range from less than 0.5 μg L^{-1} to more than 5000 μg L^{-1}. Previous study showed that the concentration of As in unpolluted fresh water typically ranges from 1.0-10.0 μg L^{-1}, rising to 100-5000 μg L^{-1} in sulfide mineralization and mining area (Smedley et al., 1996). Some reviews concerning the occurrence and distribution of As species have been made to enable researchers better understanding the behavior of As in environment (Mandal, 2002; Wilson et al., 2010; Plant et al., 2006). Seawater generally contains 1.0-8.0 μg L^{-1} As, and As(V) was assumed being the dominant species (As(V) : As(III) = 10^{26} : 1) from thermodynamic calculations (oxygenated seawater at pH of 8.1). However, in reality the ratios of As(V):

As(III) ranged from 0.1:1 to 10:1 (Johnson, 1972). Biological reduction may play an important role in affecting the distribution of As species. Arsenic is also an important constituent in geothermal fluids, ranging from 0.1 to 50 mg L^{-1}, *e.g.* up to 8.5 mg L^{-1} in New Zealand (Ritchie, 1960), 6.4 mg L^{-1} in Japan (Nakahara et al., 1978), and up to 9.2 mg L^{-1} (chapter 5) in Java. Speciation analysis of As in geothermal systems indicated that As occurred in two oxidation states, As(III) and As(V), and As(III) seemed to be the main aqueous species in hydrothermal fluids (Ballantyne and Moore, 1988; Breuer and Pichler, 2013). Organic As species such as MMA, DMA and AB were also identified in marine environment but only minor fractions were detected due to the adsorption on to suspended particles.

1.1.2 Sb

Antimony (Sb) is a trace element and the 63rd most abundant occurring element in the Earth's crust, but its crustal abundance is about one order of magnitude lower than As (Reimann et al., 2010). Sb in the aquatic environment can be originated from rock-weathering, soil runoff and anthropogenic activities. Generally, the concentrations of Sb in unpolluted water are very low, ranging from a few ng L^{-1} to a few µg L^{-1} depending on different chemical and physical conditions (Onishi, 1969; Schutz and Turekian, 1965). Sb was not well documented and often overlooked, due to its lower abundance and relative insolubility of most of its compounds. However, anthropogenic related sources, may lead to up to 100 times higher values. The U.S. Environmental Protection Agency (EPA) considers it a priority pollutant and the Council of the European Union (1998) established the maximum admissible level of Sb in drinking waters at 5.0 ug L^{-1}. Sb has two isotopes; ^{121}Sb and ^{123}Sb with the abundances of 57.21% and 42.76% respectively. It occurs in four oxidation states (-III, III, IV and V), with two oxidation states +III and +V being the predominant species in environment. Sb is thought to be chemically similar to As, as they are both metalloids and have the same oxidation states. However, previous studies have found that Sb may have quite different behavior regarding oxidation, adsorption and bioavailability (Wilson et al., 2010).

The existing forms of Sb species are different depending on pH and oxidation states (section 1.2). Compared to As species, Sb(III) in solution has a complexing properties, and can form complexation with organic ligands under acidic conditions, such as EDTA, DTPA. Distribution and speciation of Sb in freshwater and ocean water have not been

studied extensively, probably due to the lack of samples preservation methods. Sb concentration in surface marine waters was 184 ± 45 ng L^{-1} (Filella et al., 2002b), higher by a factor of 3 to 4 times higher than in fresh water. Previous studies (Mok and Wai, 1987; Shieh, 1993; Ulrich, 1998; Mok and Wai, 1990) reported that Sb(V) was the dominant species under oxic conditions. However, significant concentration of Sb(III) was also detected. Similarly, the Sb(V) was reported under anoxic conditions. This is contradicting thermodynamic equilibrium predictions. Biological activity or kinetic effects may partially explain the discrepancy but have not yet been verified (Filella et al., 2002b). Besides, methylated antimony species were monitored in a few studies but only at trace level. Sb is present in geothermal systems at substantial concentrations, ranging from 500 mg L^{-1} up to 10 wt.% (Ritchie, 1960; Stauffer and Thompson, 1984; Weissberg et al., 1979).

1.1.3 Se

Selenium (Se) has six natural stable isotopes (^{74}Se, ^{76}Se, ^{77}Se, ^{78}Se, ^{80}Se, and ^{82}Se); the most important are ^{78}Se and ^{80}Se, with natural abundances close to 50 and 24%. Se can exist in the -II, 0, IV, and VI oxidation states. Se occurs in natural waters principally in two oxidation states, Se(IV) and Se(VI). Se was introduced into aquatic environment by both natural processes (weathering or run-off from rocks) and human activity (leachate from agricultural activity, combustion) (B'Hymer and Caruso, 2006).

In contrast to arsenic, trace concentrations of selenium are essential to human and animal health. Selenoproteins, incorporated in enzymes, are essential components for cellular functions in most mammals. However, there is a fine line between low intake leading to selenium deficiency (< 40 µg d^{-1}) and copious intake leading to toxicity (> 400 µg d^{-1}) in humans (Boyd, 2011). The WHO guideline value for Se in drinking water is 10.0 µg L^{-1}. Though the Se concentration in most natural waters is less than 1.0 µg L^{-1}, occasionally much higher concentrations were found in groundwater, *e.g.* extremely high concentration of up to 1300 µg L^{-1} were detected in Colorado River catchment, USA (Engberg, 1999). Groundwaters generally contain higher Se concentrations than surface waters due to water–rock interactions (Frankenberger and Benson, 1994).

Similar to As and Sb, the existing form and distribution of Se(IV) and Se(VI) are determined principally by pH and Eh conditions, however, competitive solubility,

complexation and biological interaction may also play a part. Previous studies on Se speciation showed some difference in the Se(IV) to Se(VI) ratio. It did not follow the ratio of other redox couples (*e.g.* Fe^{2+}/Fe^{3+}) (White and Dubrovsky, 1994). This reflected the slow reaction kinetics (Measures and Burton, 1978; Plant et al., 2006). In contrast to As, the reduced form of Se(IV), is very strongly adsorbed by oxides and clays. This explains the very low concentration of Se in reducing environment and the remarkable difference in behavior of As and Se in natural environment. Se in seawater was estimated at 0.17 μg L^{-1} (Thomson et al., 2001). Detailed study on Se distribution and speciation in seawater (Cutter and Cutter, 2001) showed that Se(VI) was generally higher than Se(IV) in marine waters and the concentration of Se with depth showed surface water depletion and deep water enrichment (due to deposition and mineralization). However, a substantial fractionation of Se(IV) can also be detected if microbiological processes (converting Se(VI) to Se(IV)) are involved (Measures and Burton, 1978). Besides, organic selenide was also found in surface ocean waters but was not detected in mid- or deep waters.

1.2 Existing forms of As, Sb and Se in aqueous environment

1.2.1 As

Since the solubility, mobility, bioavailability and toxicity of As, Sb and Se are related to their oxidation states, studies concerning distribution and transformation are necessary in order to understand their behavior in the environment. Redox potential (Eh) and pH, as the most important factors controlling inorganic As, Sb and Se species in natural waters, are used widely to analyze and predict their distributions under different conditions (Wilson et al., 2010).

Fig. 1.1 shows the Eh-pH diagram for As-O_2-H_2O system. It can be seen that in extremely acidic (pH < 2) and alkaline (pH > 12) conditions, H_3AsO_4 and AsO_4^{3-} were dominant. Under oxidizing conditions with pH ranging from 2 to 7, $H_2AsO_4^-$ is dominant, whereas at higher pH (from 7 to 12), $HAsO_4^{2-}$ is the main existing form.

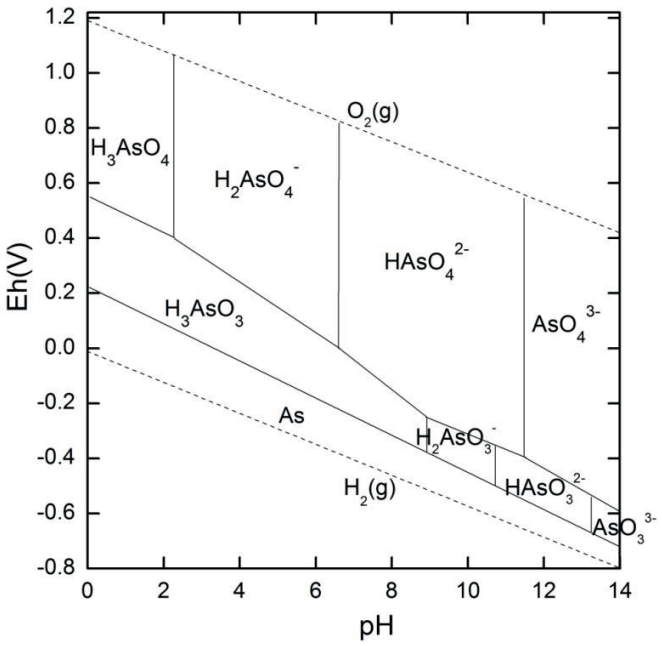

Fig. 1.1 Eh-pH stability diagram for As-O_2-H_2O system at 25 °C, 1bar. Dashed lines indicate environmental limits imposed by the dissociation of water to H_2(g) and O_2(g). (Brookins, 1988)

On the other hand, under reducing conditions with a wide pH range of 0 to 9, As(III) exists exclusively as non-charged H_3AsO_3. The lack of charge on the As(III) species compared to the successive deprotonation of As(V) species implies less charge dependence associations with solid phases, such as clay minerals and (oxy)hydroxides in soils. Thus it can be concluded that As(III) species are more mobile than As(V) in a wide pH range (Bhattacharya et al., 2002). While under alkaline conditions, As(V) exists as negatively charged oxyanions, such as $H_2AsO_3^-$ at pH of 9 - 10, $HAsO_3^{2-}$ at pH of 11 - 13 and AsO_3^{3-} at pH higher than 13. In addition, numerous studies have shown that As and Sb inorganic species predominate over organic species in most environmental systems (Andreae et al., 1981; Ellwood and Maher, 2002; Sun et al., 1993). It is worth noting that Fig. 1.1 is a simplified illustration of species distribution, without other elements involved. In fact other variables could also influence the behavior of As species in a more complex system. With addition of Fe, As would co-precipitate with Fe-(oxy)hydroxides, *e.g.* as the hydrated iron arsenate mineral scorodite ($FeAsO_4 \cdot 2H_2O$) (Mok and Wai, 1990). While at the presence of extremely high concentration of reduced S, the formation of dissolved As-sulphide species can be significant, *e.g.* (co)precipitation as orpiment (As_2S_3), realgar (AsS) or other sulphide minerals under reducing acidic conditions (Bowen, 1979). Therefore, high concentrations of dissolved

As were not expected in waters with a high concentrations of free sulphide (Moore et al., 1988).

1.2.2 Sb

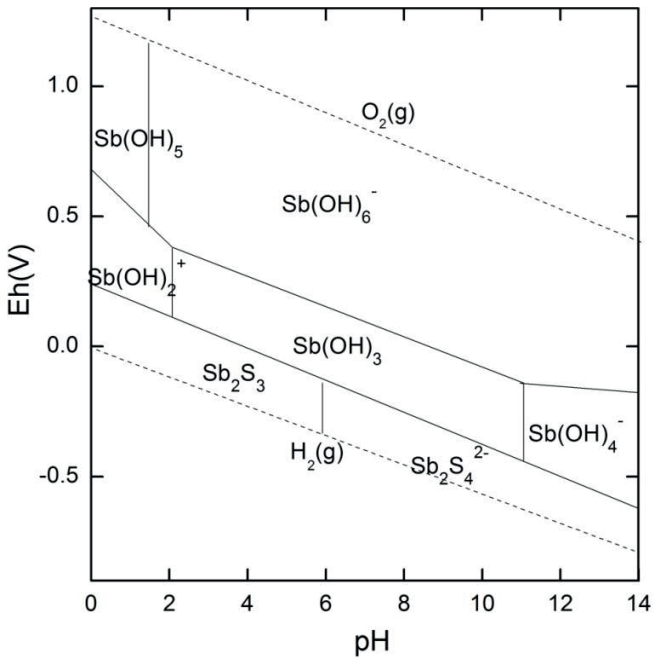

Fig. 1.2 Eh-pH stability diagram for Sb-S-H_2O system at 25 °C, 1bar with a dissolved Sb of 10^{-8} mol L^{-1} and S of 10^{-3} mol L^{-1}. Dashed lines indicate environmental limits imposed by the dissociation of water to $H_2(g)$ and $O_2(g)$. (Filella et al., 2002b)

For Sb (Fig. 1.2) the Eh-pH diagram shows that Sb(V) exclusively exists as negatively charged $Sb(OH)_6^-$ (the coordination of Sb(V) with oxygen is octahedral) in a wide pH range from acid to alkaline, which is different from As(V). As has been mentioned As(V) was deprotonated in successive steps in a similar pH range. Under extremely acidic conditions (pH < 1), Sb(V) exists as non-charged $Sb(OH)_5$. As for Sb(III), non-charged $Sb(OH)_3$ exists in a wide pH range from 2 to 11 with pKa = 11.9 (Table 1.1). Similar to As, the mobility of Sb (III) is higher than Sb(V). Besides, the exclusive existing form of Sb(V) (as $Sb(OH)_6^-$) but successive protonation of As(V) in a wide pH range from acidic to alkaline indicated that the binding of As(V) to particulate matter in oxygenated systems is more complicated than that of Sb(V). Previous studies have shown that Sb(V) formed mainly outer sphere complexes with Fe-(oxy)hydroxides, while As(V) formed

inner sphere complexes (Goldberg and Johnston, 2001; Ona-Nguema et al., 2005; Leuz, 2006).

On the other hand, Sb(III) exists as positively charged $Sb(OH)_2^+$ under extreme acidic conditions (pH < 2) and negatively charged $Sb(OH)_4^-$ under alkaline conditions (pH > 11). In the wide pH range from 2 to 11, Sb(III) exists as dissolved $Sb(OH)_3$. This diagram was obtained based on environmentally relevant concentrations: Sb of 10^{-8} mol L^{-1} and dissolved S of 10^{-3} mol L^{-1}. According to this result, under reducing conditions at presence of S, stibnite Sb_2S_3(s) is formed at low to neutral pH range. At higher pHs, $Sb_2S_4^{2-}$ was formed instead of Sb_2S_3. However, when the concentration of Sb in the environment exceeds 10^{-6} mol L^{-1}, Sb(III) would be present as solid species, *e.g.* in the form of polymorphs senarmontite and valentinite (Sb_4O_6), instead of $Sb(OH)_3$(s) under acidic to alkaline and moderately reducing to moderately oxidizing conditions (Vink, 1996). As for Sb(V), the ionic species SbO_3^- ($Sb(OH)_6^-$) occupies a large range under oxidizing conditions from acidic to alkaline conditions, indicating a relatively high mobility. Noteworthy, Sb(V) was previously thought to be immobile under oxidizing conditions and existed in the form of Sb_2O_5 (Brookins, 1986, 1988).

1.2.3 Se

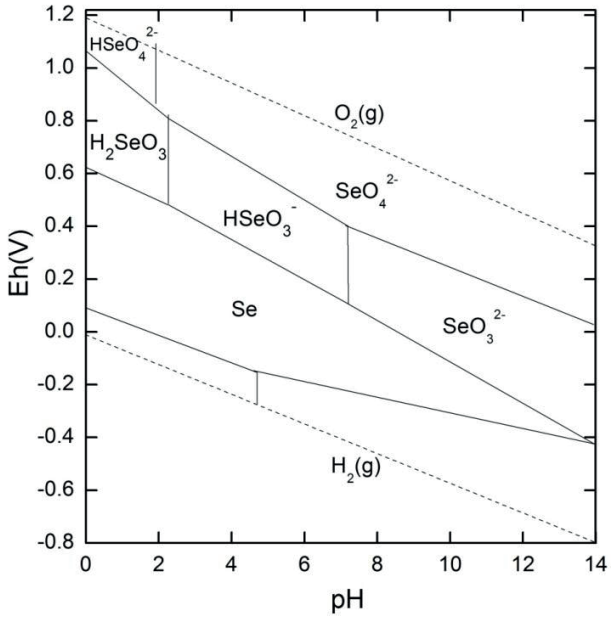

Fig. 1.3 Eh-pH stability diagram for As-O_2-H_2O system at 25 °C, 1bar. Dashed lines indicate environmental limits imposed by the dissociation of water to H_2(g) and O_2(g). (Brookins, 1988)

Similar to As and Sb, Se also is a redox sensitive element. Sulfur and iron compounds play an important part in the transportation of Se. Se occurs in water solutions principally in two oxidation states, Se(IV) and Se(VI). For Se(VI), SeO_4^{2-} mainly exists under oxidizing condition in a pH range of around 2 to extremely basic conditions. $HSeO_4^{2-}$ exists at a pH less than 2. It can be seen in table 1.1 that H_2SeO_4 is an acid with a pKa of 2.0. For Se(IV) $HSeO_3^-$ and SeO_3^{2-} were the main existing forms under reducing conditions in a wide pH range from 2 to 14. H_2SeO_3 is formed under very acidic conditions (pH < 2). In soils and sediments, elemental Se dominates under strong reducing conditions. Considering the main existing form of Se(VI) and the successive protonation of Se(IV), Se(IV) is generally more available and more mobile than Se(VI). Previous study of Se distribution and speciation for seawater showed that the concentration of Se(VI) was generally higher than Se(IV) (Cutter and Cutter, 2001).

Table 1.1. Equations and pKa values for inorganic As, Sb and Se species.

As(V)	**pKa**
$H_3AsO_4 + H_2O = H_2AsO_4\text{-} + H_3O^+$	2.20
$H_2AsO_4^- + H_2O = HAsO_4^{2-} + H_3O^+$	6.97
$HAsO_4^{2-} + H_2O = AsO_4^{3-} + H_3O^+$	11.53
Sb(V)	
$Sb(OH)_5 + 2H_2O = Sb(OH)_6^- + H_3O^+$	2.72
Se(VI)	
$H_2SeO_4 + H_2O = HSeO_4^- + H_3O^+$	2.0
As(III)	
$H_3AsO_3 + H_2O = H_2AsO_3^- + H_3O^+$	9.22
$H_2AsO_3^- + H_2O = HAsO_3^{2-} + H_3O^+$	12.13
$HAsO_3^{2-} + H_2O = AsO_3^{3-} + H_3O^+$	13.4
Sb(III)	
$Sb(OH)_3 + 2H_2O = Sb(OH)_4^- + H_3O^+$	11.9
Se(IV)	
$H_2SeO_3 + H_2O = HSeO_3^- + H_3O^+$	2.6
$HSeO_3^- + H_2O = SeO_3^- + H_3O^+$	8.3

1.3 Interferences in plasma

When analyzing As and Se using ICP-MS, the main difficulties are interferences. There are many spectral and non-spectral interferences for As and Se determinations. Spectral interferences mainly occur as poly atomic species, such as $^{35}Cl^{40}Ar$ on ^{75}As, $^{40}Ar^{40}Ar$ on ^{80}Se and $^{81}Br^{1}H^{+}$ on ^{82}Se (table 1.2). These interferences could be caused by plasma gas ions (*e.g.* $^{40}Ar^{40}Ar$), interaction of plasma gas with other species (from reagents or sample) (*e.g.* $^{35}Cl^{40}Ar$) and sample matrix (*e.g.* $^{81}Br^{1}H^{+}$). Generally four strategies were used to handle these interferences.

1) Selection of interference-free isotopes for analysis and high resolution mode of detection (if possible for instrument). *e.g.* Thermo element 2/XR sector field ICP- MS provides three resolution modes: low resolution mode (> 300), medium (> 4000) and high (> 10000). It can analyze almost all kinds of samples and their matrices (seawater, hydrothermal solution, leachates etc.) free of interferences. Thus, for As measurements, as it is a mono-isotopic element and a resolution of at least 7775 was needed to separate $^{35}Cl^{40}Ar$ and ^{75}As (Jakubowski et al., 2011). Obviously, the high resolution mode of element 2/XR was sufficient. As for Se measurements, the isotope of ^{78}Se was monitored in high resolution mode to avoid interferences. However, the using of high-resolution mode implies a loss of signal intensity, which elevates the detection limit accordingly, thus retards the measurement with more accuracy and precision, especially for those elements of low abundance (*e.g.* ^{78}Se, with an abundance of 23.6%).

2) Using mathematical equation to correct interferences. For the ICP-MS, many correction equations are built to facilitate automatic corrections of certain isobaric or polyatomic interferences. For As the most common equation is:

Corrected ^{75}As signal = total signal in mass 75 – (3.127 x (signal in mass 77 – (0.815 x signal in mass 82)))

e.g. ^{75}As (corrected) = $^{75}As – (3.127 \times (^{77}Se – (0.815 \times ^{82}Se)))$

However, this equation was based on two assumptions: a) all signals in mass 82 are from Se and b) after subtraction of ^{77}Se contribution on mass 77, the remaining signals on mass 77 are due to $^{37}Cl^{40}Ar$. The problem is that if the samples contain high bromine,

the signals in mass 82 are a combination of ^{82}Se and $^{81}Br^{1}H$. As a result, the correction equation would produce large bias. As for ^{82}Se, the common used correction equation is:

Corrected ^{82}Se signal = total signal in mass 82 – (0.007833 x signal in mass 83) – (0.00187 x signal in mass 79)

e.g. ^{82}Se (corrected) = ^{82}Se – (0.007833 x ^{83}Kr) – (0.00187 x ^{79}Br)

Obviously, this equation was also matrix dependent, as ^{79}Br was monitored. There is no universal method for dealing with interferences in ICP-MS. It seems wise to always monitor more than one isotope (if possible), even if the other isotopes are less abundant.

3) Using chromatography to remove Cl-interferences. Since in aquatic environments Cl and As species exist as anions, it is possible to use anion exchange chromatography to remove Cl-related interferences, *e.g.* $^{40}Ar^{35}Cl^{+}$ on ^{75}As. In our previous work (Wu and Pichler, 2014) the potential interference of $^{40}Ar^{35}Cl^{+}$ was solved using a Hamilton PRX-X100 anion exchange column, as Cl^{-} eluted out at a different retention time from As(III) and As(V).

4) Using other techniques such as “collision / reaction cell”. The collision / reaction cell technique known as Elan DRC (I, II, e) was introduced by Perkin-Elmer, which is a chamber placed between the single lens optics chamber and the mass analyzer chamber of ICP-MS for eliminating isobaric interferences. The chamber has a quadrupole and can be filled with reaction (or collision) gases (HN_3, CH_4, He O_2 or H_2). The gas reacts with the introduced sample, and eliminates some of the interferences. The mechanism is based on neutralization of exchange reaction between interfering ions and reaction gas, producing different m/z^{+}, *e.g.* methane was used for As and Se analysis (Komorowicz and Barałkiewicz, 2011).

However, the application of high-resolution mode and collision / reaction cell can both lead to drop of signal intensity. There is no universal method for dealing with interferences in ICP-MS. A successful strategy requires a full understanding of the technique and detailed knowledge of sample matrices.

Table 1.2 Spectral interferences in measurement of As, Sb and Se.

	isotopes	interferences
As	^{75}As	$^{40}Ar^{35}Cl^{+}$
Se	^{74}Se	$^{37}Cl^{37}Cl^{+}$
	^{76}Se	$^{12}C_6{}^{1}H_4{}^{+}$
		$^{36}Ar^{40}Ar^{+}$
	^{77}Se	$^{37}Cl^{40}Ar^{+}$
		$^{12}C_6{}^{1}H_5{}^{+}$
		$^{12}C_5{}^{1}H^{16}O^{+}$
	^{78}Se	$^{12}C_6{}^{1}H_6{}^{+}$
		$^{38}Ar^{40}Ar^{+}$
	^{80}Se	$^{1}H^{79}Br$
		$^{40}Ar^{40}Ar^{+}$
	^{82}Se	$^{1}H^{81}Br$
		$^{40}Ar^{40}Ar^{1}H_2{}^{+}$
Sb	^{121}Sb	$^{105}Pd^{16}O^{+}$
	^{123}Sb	$^{94}Zr^{16}O_2$

1.4 Necessity for speciation of As, Sb and Se redox couples

Among the redox sensitive elements, such as As, Sb and Se, inorganic species are the most abundant and most toxic in environment. Numerous studies have shown that toxicity, redox stability, adsorption, mobility and biogeochemical cycling are related to their inorganic species. Besides, their behavior is quite different from each other and inter-influence can occur, such as competitive adsorption on an iron-(oxy)hydroxide surface. Thus simultaneous speciation of these species is necessary in order to better understand their behavior in the environment. Besides, the ratios of these redox couples were proved to be a promising tool for geochemistry. However, up to date, the simultaneous determination of these species remains a great challenge. Furthermore, the inability of preserving the distribution of As, Sb and Se species retarded further investigation.

1.5 Detector

Various detection systems have been widely used for As, Sb or Se determination, such as ultra violet (UV) detection (Jaafar et al., 2009; Koshcheeva et al., 2009), potentiometry and conductometry such as polarography, cathodic stripping voltammetry (CSV) and anodic stripping voltammetry (ASV) (Smichowski et al., 1998; Domínguez-Renedo et al., 2009), AFS (Gregori et al., 2005; Price and Pichler, 2005), ICP-AES (Chausseau et al., 2000) and ICP-MS. However, each type of detection system has its advantages and limitations, *e.g.* UV and potentiometry and conductometry systems are low-cost and easy to operate but their limitations are not low enough to meet the trace or ultra-trace level determination. ICP-AES has the advantages of high flexibility and satisfactory accuracy and precision over a broad range of concentrations. Meanwhile, dissolution of solids may bring about problems, and the detection limits are usually not low enough for trace elements, like As, Sb and Se. AFS coupled to HG, however, is a well-established technique, with great sensitivity for As and Sb, even comparable to ICP-MS. In addition, the purchase and operating costs are low. However, HG technique is only suitable for those elements which form volatile covalent hydrides, *e.g.* HG-AFS is not applicable for simultaneous speciation of Se species due to its inability of forming Se(VI)-hydride. Thus, the basic speciation includes two replicate measurements, one for total concentration and the other for one of the inorganic species. The concentration of the other species was obtained by subtraction of the two. However, the drawback is this procedure overlooked the presence of other species, such as various organic species. For ICP-MS, the strong points are: low detection limits for trace element analysis; excellent possibilities for correcting spectral interferences; high resolution detection mode for almost all elements free of interferences (sector field ICP-MS). But, the weak points are also obvious: accuracy and precision are less than ICP-AES for some particular elements; the costs are much higher than for ICP-MS and special operation skills may be necessary (Rommers and Boumans, 1996).

Generally, there is no universal detector, which is ideal for all elements determination in a wide concentration range free of interferences. They may supplement and complement each other under different conditions. The choice of detectors must be based on various analytes and analytical requirements. As for the elements of our interest (As, Sb and Se), the sector field-ICP-MS seems the best choice, because it allowed simultaneous and interference-free (*e.g.* complete separation of ^{75}As from $^{35}Cl^{40}Ar$ and ^{80}Se from $^{40}Ar^{40}Ar$)

determination at trace level (Wu and Pichler, 2014). Regardless which detection system was used, the detector itself was not capable of separating different species of a given element (*e.g.* As(III) and As(V), Sb(III) and Sb(V) and Se(IV) and Se(VI)) in plasma, though ICP-MS provides "pseudo" simultaneous detection of different masses. Thus for speciation analysis, a separation technique (*e.g.* selective extraction or chromatography based separation) is needed before introduction in detection system. In addition, the combination of HPLC to ICP-MS provides another possibility of dealing with isotopic mass interference. *E.g.* the common interference of $^{35}Cl^{40}Ar$ on ^{75}As in direct determination by ICP-MS can be solved by chromatography, as the species of $^{35}Cl^{40}Ar$ and ^{75}As can elute out at different retention times from chromatographic column and thus are subsequently introduced in plasma separately.

2. Speciation methods for As, Sb and Se species (a review)

Speciation is defined as analytical identification and quantitative determination of different chemical forms of the elements present in a sample (Templeton et al., 2000). However, selective determination of each species in the presence of other chemical forms of the same element is usually impracticable. Thus, the separation and detection of various analytes of a certain element or even various species of more than two elements is necessary. Basic separation includes non-chromatographic methods, i.e. electrokinetic separation methods (Capillary electrophoresis (CE) (Koellensperger et al., 2002; Sun et al., 2002), supercritical fluid chromatography (SFC) and solid phase extraction (SPE) (Wu et al., 2009, 2011; Planer-Friedrich et al., 2006), and chromatographic methods, i.e. gas chromatography (GC), high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC).

2.1 Non-chromatographic speciation

Though great progress has been made in hyphenated technique in speciation, other chemistry-based separation procedures are still important, *e.g.* liquid-liquid extraction (LLE), liquid-phase microextraction (LPME), cloud-point extraction (CPE), solid-phase extraction (SPE), capillary electrophoresis (CE) and hydride generation (HG). These techniques provide quantitative information on specific chemical forms of some elements in many types of samples at reduced cost and time.

2.1.1 Liquid-liquid extraction (LLE)

Liquid–liquid extraction (LLE), also known as solvent extraction and partitioning, is a method to separate various species based on their relative solubility in two different immiscible liquids, usually water, and an organic solvent. This technology is extremely simple at low cost. Great improvement has been achieved based on LLE. Recently, a micro-extraction technique-dispersive liquid-liquid micro-extraction (DLLME), based on a ternary solvent system was developed. An appropriate mixture of extraction solvent and disperser solvent is rapidly injected into an aqueous sample, thus a cloudy solution is formed. Then the analyte in the sample is transferred to the fine droplets of the

extraction solvent. Phase separation is performed by centrifugation. In an As speciation analysis study (Escudero et al., 2013), selective separation of As(III) was achieved by chelation with sodium diethyldithiocarbamate (DDTC) followed by dispersion with 1-octyl-3-methylimidazolium hexafluorophosphate. As(III) was then extracted with a packed micro-column and subsequently measured with electrothermal atomic absorption spectrometry (ETAAS). The concentration of As(V) was deduced by the difference between total inorganic As and As(III). In another report of As and Sb speciation in waters (Rivas et al., 2009), As(III) and Sb(III) were complexed with ammonium pyrrolidine dithiocarbamate at first and then mixed with carbon tetrachloride (extraction solvent) and methanol (disperser solvent). After centrifugation As(III) and Sb(III) were extracted in the organic phase and measured with ETAAS, while As(V) and Sb(V) remained in the aqueous layer.

2.1.2 Liquid-phase microextraction (LPME)

LPME is a simple, and highly sensitive technique for sample pretreatment before trace analysis of analytes from complex matrices. It is a miniaturized implementation of conventional liquid-liquid extraction in which only a few µLs of solvents are used. Some LPME-based methods for As, Sb or Se speciation have been developed. *e.g.* single-droplet micro-extraction (SDME) and hollow fiber liquid-phase microextraction.

a) Single-droplet microextraction (SDME)

The basic procedure of SDME is: 1) a precious micro-syringe was used to draw up extraction solvent (less than 3 µL, typically organic); 2) the micro-syringe was slightly expelled to make sure that a drop (1-3µL) of extraction solvent suspended at the tip; 3) expose the droplet to sample under optimized conditions (*e.g.* temperature and extraction time); 4) the droplet is retracted and transferred for further determination. Although originally developed for organic analytes extraction, SDME has been proved to be also highly effective for pre-concentration and speciation of trace metals. Fan (2007) developed a speciation method for Sb inorganic species in water samples using SDME followed by analysis using ETAAS. In the method *N*-Benzoyl-*N*-phenylhydroxylamine (BPHA)-chloroform single drop was used, where BPHA worked as complexing agent. Total concentration of Sb was determined after pre-reduction (Sb(V) to Sb(III)) by L-

cysteine. Sb(V) was calculated by subtraction. The detection limits were 8.0 ng L^{-1} for Sb(III) and 9.2 ng L^{-1} for total Sb, respectively.

Another type of improved SDME is head-space single-droplet microextraction (HS-SDME). The biggest difference is in step 3: in HS-SDME the drop was not directly exposed to the sample but in the sample head-space. The volatile species would be volatilized under certain temperature into headspace and extracted to the drop. After the species between head space and the drop achieve equilibrium, the micro-drop was retracted for determination. Chamsaz et al. (2003) successfully used this method for As analysis. An organic solvent (a mixture of pyridine and benzyl alcohol, 1:3 v/v) with dissolved silver diethyldithiocarbamate (AgDDC) was used for extracting As species. As species in aqueous samples were converted to As-hydrides using sodium tetrahydroborate ($NaBH_4$). During 7 min extraction at 35 °C, the As-hydrides reacted with AgDDC and were extracted by a 4 µL micro-drop suspended in the tip of micro-syringe. The determination was carried out on a GFAAS and the detection limit for As (total) was 45 pg mL^{-1}.

b) Hollow fiber liquid-phase microextraction (HFLPME)

HFLPME is a membrane-based separation technique, which was also referred to micro-porous membrane extraction (Fig. 2.4). The basic extraction process includes: 1) conditioning of the hollow fiber (make the hydrophobic porous membrane impregnated with organic solvent); 2) injection of a specific volume of the solvent into the conditioned hollow fiber using micro-syringe; 3) immersing the hollow fiber into sample (the analytes would partition from the aqueous sample into the organic solvent); 4) retracting of the extracted sample for analysis. This method is suitable for extraction of species with large partitioning coefficients in the organic solvent. It has been used for speciation of inorganic Se species in natural water samples (Xia et al., 2006). Chloroform was used as organic solvent and ammonium pyrrolidine dithiocarbamate (APDC) was used as chelating agent. During extraction Se(IV) was extracted by the organic solvent due to the formation of a Se(IV)-PDC complex, while Se(VI) remained in the solution as free species. The reported detection limits are: 0.50 pg mL^{-1} for Se(IV) and 2.7 pg mL^{-1} for Se(VI).

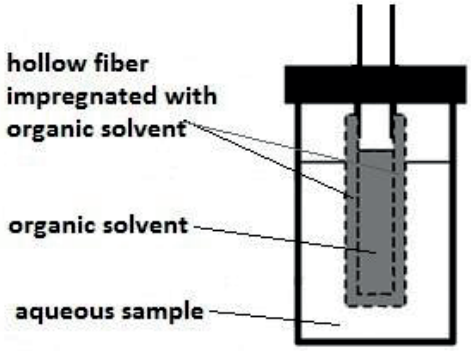

Fig. 2.4 Scheme for hollow fiber liquid phase micro-extraction (HFLPME).

2.1.3. Cloud-point extraction (CPE)

Another separation strategy similar to LLE is cloud-point extraction (CPE), based on the selective extraction of analytes by non-ionic surfactant. When heated to a certain temperature (known as could point) the non-ionic surfactant would become turbid. Above this temperature, the isotropic micelle solution separates into two phases: the surfactant-rich phase with small volume, and the diluted aqueous phase where the surfactant concentration is very low (close to the critical micelle concentration). The analytes (or analyte-chelates, generated by addition of chelation agents) would be extracted preferentially by the surfactant-rich phase (Stalikas, 2002; Paleologos et al., 2000). Complete phase separation can be obtained after centrifugation. A method for simultaneous speciation analysis of inorganic Sb and Se in water samples was developed (Li et al., 2008) based on the fact that Sb(III) and Se(IV) could form complexes with diethyldithiocarbamate (DDTC) at a pH of 6. The complexes were extracted into the surfactant-phase of octylphenoxypolyethoxyethanol (Triton X-114) when heated in thermostated water bath of 30 °C, whereas Sb(V) and Se(VI) remained in the aqueous solution. The extracted Sb(III) and Se(IV) were subsequently determined by ETV-ICP-MS. Total concentration of Sb and Se was determined by the same protocol after pre-reduction by L-cysteine and the concentration of Sb(V) and Se(VI) was obtained by subtraction. The limits of detection (LODs) were 0.05 μg L^{-1} for Se(IV) and 0.03 μg L^{-1} for Sb(III).

2.1.4 Solid-phase extraction (SPE)

Solid-phase extraction can be used to isolate analytes of interest from a wide variety of matrices. SPE has been frequently used as a technique for speciation analysis. This is because SPE avoids usage of large amounts of organic solvents and provides larger pre-concentration factors and lower detection limit. The basic principle is: when sample passes through stationary phase, the analytes in the sample interact and retain on the sorbent of stationary phase. Other species would pass through the solid phase and are then discarded. The desired analytes are eluted with a kind of solvent and then detected. Some novel speciation methods based on SPE have been developed. Ben Issa et al. (2010, 2011) combined a strong base anion exchange resin (SBAE) and two hybrid (HY) resin: HY-Fe (based on behavior of hydrated iron oxide particles on As species) and HY-AgCl (adsorbent for inorganic As(III) and As(V)) for inorganic As species (As(III) and As(V)) and organic As species (MMA and DMA). Separation of these species was achieved based on the following: 1) at pH < 8, SBAE resin separated As(V) from As(III) by retaining As(V) and allowing As(III) to pass through. So As(III) can be measured in the effluent. 2) within a wide pH range from 5 to 11, HY-Fe resin retained both As(III) and As(V), except for DMA. Thus, DMA could be measured. 3) HY-AgCl resin at pH near 9 retained both inorganic As(III) and As(V), but allowed organic As species of MMA and DMA to pass through, which made detection of organic As species possible (Fig. 2.5).

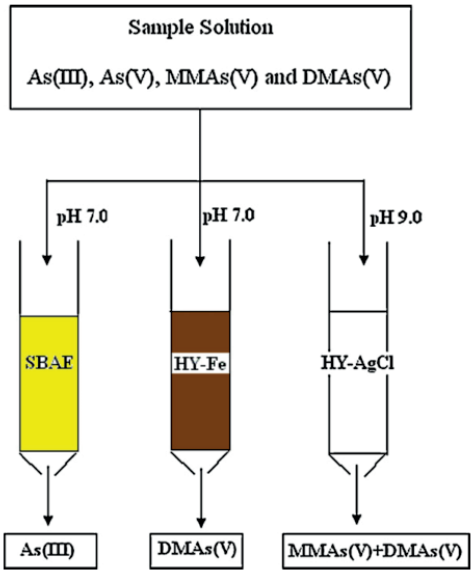

Fig. 2.5 Scheme for selective separation of As species in water samples using SBAE, HY-Fe and HY-AgCl resins (from (Ben Issa et al., 2011)).

Wu et al. (2011) achieved simultaneous speciation of inorganic As and Sb species in water samples with on-line SPE using single-walled carbon nanotubes (SWCNTs) micro-column. The micro-column was simply made by joining two micropipette tips: the upper part of a micropipette tip was cut off and inserted into another one. SWCNTs was put into the micro-column and a bit of glass wool was placed at both ends to avoid loss of sorbent during elution (Fig. 2.6).

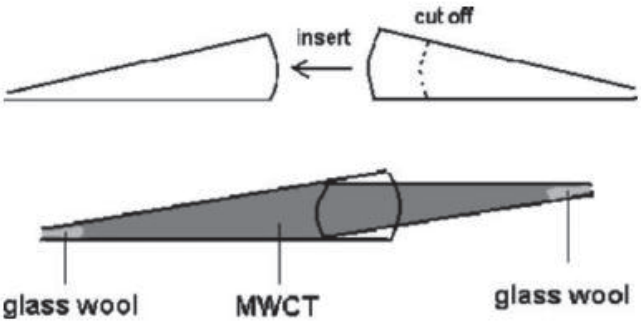

Fig. 2.6 Scheme of SWCNTs packed micro-column (Wu et al., 2009).

Ammonium pyrrolidine dithiocarbamate (APDC) was used to complex As(III) and Sb(III). When samples and APDC passed through the micro-column, complexes of As(III)-APDC and Sb(III)-APDC were formed and retained on the adsorbent. The complexes were then eluted out by HNO_3 (20%, v/v) and measured by hydride generation-double channel atomic fluorescence spectrometry (HG-(DC)AFS). Total As and Sb were determined after As(V) and Sb(V) were reduced by thiourea. Thus, As(V) and Sb(V) were obtained by subtraction of the two values.

2.1.5 Capillary electrophoresis (CE)

Capillary electrophoresis (CE) has been proven to be a potential powerful method for speciation. The principle for separation of various species is: charged analytes would migrate toward the opposite electrode when an electric field is applied. Since various analytes have different electrical mobility, they can be separated during migration. Liu et al. (2013) successfully separated 10 As species using capillary electrophoresis (CE) coupled with ICP-MS, including inorganic As(III) and As(V), and organic As species of MMA, DMA, AC and AB. The separation was achieved on a 100 cm length × 50 μm ID fused-silica capillary. The detection limits of the ten arsenic compounds ranged from 0.9 to 3.0 ng g^{-1}. Another study of simultaneous speciation of As, Se, Sb and Te species in

waters and soil extracts using CE and UV detector was made by Casiot et al. (1998). The separation was achieved within 5 min at electrolyte pH of 11.2. However, relatively high detection limits were obtained, from 13 μg L^{-1} for Se(VI) to 509 μg L^{-1} for Te(IV), due to using a low-sensitivity UV detector. Generally, it can be seen that pH plays an important part in species speciation using CE. The pH of the electrolyte can directly influence the electrophoretic mobility of the analytes, because the dissociation (dissociation constant of As, Sb and Se species were listed in Table 1.1) and ionization capability of the desired species are various under different pH values. *e.g.* As(V) and Se(VI) would migrate faster than As(III) and Se(IV), due to their low pKa and two negative charges in a wide pH range.

However, a special interface for coupling CE with ICP-MS is needed. The first reason is that CE has a low flow rate of less than 1μL min^{-1}. This requires the use of a very low uptake rate nebulizer for ICP-MS to ensure high-transport efficiency and relatively high concentration of analyte brought into plasma. The second problem is the electrical connection. As is known, for a regular CE both ends of the fused silica capillary were submerged or in contact with two buffer reservoirs. Thus when CE was coupled with ICP-MS, the capillary must be connected electrically, and meanwhile still introduce buffer and analytes into nebulizer to produce a uniform aerosol for detector. Great effort has been made to improve the designs of CE interfaces, including: usage of sheath electrolyte (with constant sheath liquid flow rate) to close the electric circuit and addition of a “make-up” buffer (Majidi and Miller-Ihli, 1998a; Prange and Pröfrock, 2005; Lu et al., 1995; Taylor et al., 1998). However, due to the inherent complexity, many errors may still arise when using CE coupled with ICP-MS (Majidi and Miller-Ihli, 1998b).

2.1.6 Hydride generation (HG)

Hydride generation, as one of the most commonly used non-chromatographic speciation techniques for elements at trace level, was often coupled with AAS or AFS, and further coupled with HPLC for multi-species speciation, such as As, Sb and Se. This method was based on the fact that the analytes would form covalent hydrides after introduction into the atomization systems. Then, after liquid-gas separation, analytes could be detected in gas phase. The formation of covalent hydrides significantly improves the sensitivity and lowers the detection limits by several orders of magnitude in comparison to conventional nebulization. HG has a lot of advantages, such as: 1) easily being

coupled with a variety of additional detection techniques. *E.g.* ICP-AES, ICP-AFS and ICP-MS. 2) interference free determination of As (*e.g.* $^{40}Ar^{35}Cl$ for ^{75}As) and Se (*e.g.* $^{40}Ar^{40}Ar$ for ^{80}Se) isotopes. As is known, isotope interferences are problematic for As and Se measurements using ICP-MS, due to the very closeness of masses between desired species and interference. 3) possible selective determination of species. *E.g.* for Se and Te, only tetravalent oxidation states can form hydrides, so this species can be detected directly. For As and Sb on the other hand, though both oxidation states (As(III) and As(V), Sb(III) and Sb(V)) can form hydrides, trivalent oxidation states of As and Sb can be determined solely by controlling pH conditions. In addition, As and Sb redox couples can be separated by further coupling with a chromatographic column. Total concentration of these elements can be determined after reduction. Sodium borohydride ($NaBH_4$) is the most frequently used reducing agent. However, the biggest drawback of HG technique is that for simultaneous speciation of more than two elements (*e.g.* simultaneous speciation of redox couples of As, Sb and Se) in one analysis run, HG is useless.

In previous studies HG technique has been widely used for As, Sb or/and Se analysis. Although, HG was mainly used coupled to AAS or AFS, researchers have studied the possibility of coupling HG to ICP-MS, and gratifying results were obtained. Hou and Narasaki (1999) developed a speciation method for Sb inorganic species in waters using HG-ICP-MS. The selective separation of Sb(III) by HG was achieved at a pH of a 5.5, due to that Sb(V) can not form hydride with pH above 4.0, whereas Sb(III) can. Total concentration of Sb was determined after pre-reduction with potassium iodide solution (KI). Sengupta and Dasgupta (2009) reported an automated hydride generation (AHG) - ICP-MS method for total As analysis. According to an investigation of the reaction time in HG and the relative response of different As species, they found that a substantial reaction time of 60 s prior to release of formed As-hydride to ICP-MS resulted in essentially identical signal intensity for all four As species: As(III), DMA, MMA and As(V). This provided the possibility of direct determination of total As from complex environmental samples without pretreatment (convert all forms of As into As(III)).

Another difficulty for multi elements simultaneous determination using HG-ICP-MS is finding appropriate reductants. *E.g,* for simultaneous detection of total concentration of As, Sb and Se, the first step was to convert all species with various states to lower oxidation states (As(III), Sb(III) and Se(IV)), as Se(VI) can not form hydride. However,

the commonly used reducing agents, such as iodide or bromide, L-cysteine and thiourea, can all reduce Se(IV) to elemental Se which is not able to form hydride as well. Bowman et al. (1997) developed a procedure for simultaneous detection of As, Sb and Se using HG-ICP-MS. The method involved an off-line pre-reduction for converting Se(VI) into Se(IV), combined with an on-line reduction of As(V) and Sb(V) to trivalent state with thiourea. Although thiourea could also slightly reduce Se(IV) to Se, the conversion was slower than caused by iodide.

2.2 High-performance liquid chromatographic (HPLC) speciation

The principle of separating species with liquid chromatography was demonstrated in Fig. 2.7. Various analytes pass through the stationary phase of column and generate different velocity due to different adsorption abilities, solubilities or other properties between mobile and stationary phases. Finally various analytes are separated in column and eluted out at different retention times. Liquid chromatography, like anion exchange (AEX), cation exchange (CEX), ion exclusion (IEC), and ion pair chromatography (IPC), coupled to a sensitive detector (*e.g.* AFS, ICP-OES and ICP-MS) have been used for As, Sb or Se speciations. HPLC is more qualified for separation of naturally non-volatile As, Sb and Se species. These species are not stable if heated to the required temperature to keep them in gas phase. However, gas chromatography (GC) was qualified for these volatile organic species.

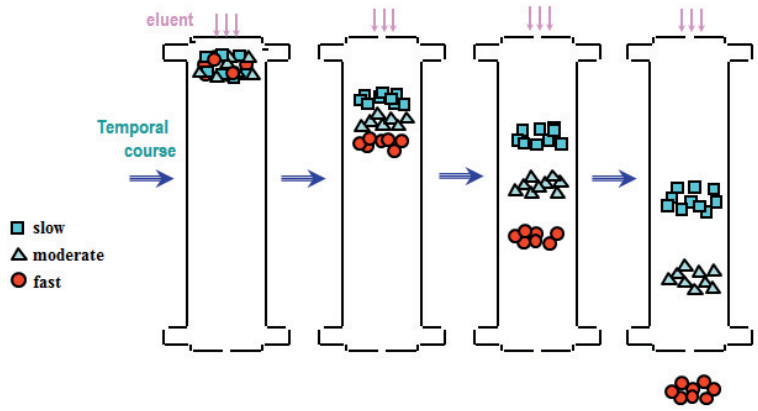

Fig. 2.7 Scheme of principle of liquid chromatography.

2.2.1 As speciation

As speciation using HPLC, has been well reviewed recently by Komorowicz and Barałkiewicz (2011) and Ammann (2011). ICP-MS was the most widely used detector for As species determination due to its high sensitivity, wide linear dynamic range and it can easily be combined to many separation techniques. The coupling of ICP-MS with liquid chromatography allows separation, identification and quantification of As species in just one analysis run. As for separation of various As species, the key factors are pH, mobile phase, and the type of chromatography. Because, As species vary under different pH and Eh conditions (section 1.2). Thus the choice of chromatography and mobile phase needs to be based on this. Fig. 2.8 shows the choice of various types of chromatography.

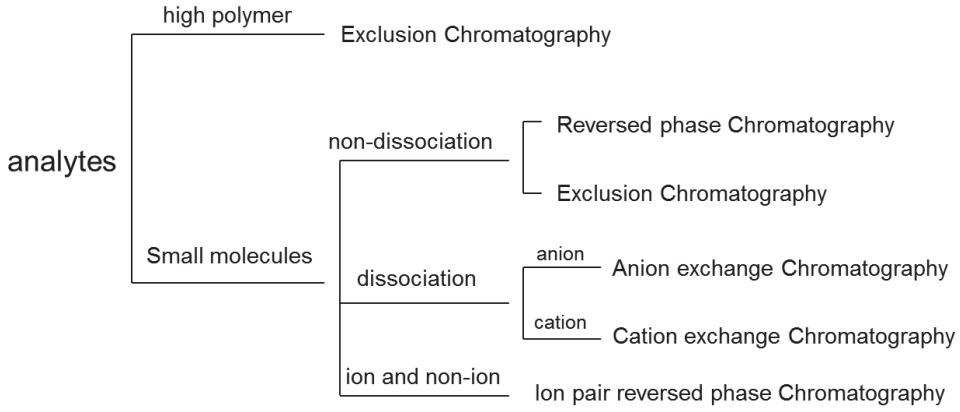

Fig. 2.8 Choice of various chromatography.

a) Reversed-phase and ion-pair chromatography

Both, simple reversed-phase (with an aqueous mobile phase, and probably a kind of organic modifier) and ion-pair reversed-phase chromatography (a counter ion is added to the mobile phase), are used for speciation analysis of ionic species, as well as for uncharged molecular species of As, Sb and Se. Commonly used ion-pair reagents are long-chain alky ions, such as alkyl sulfonates, or tetraalkylammonium salts. Ion-pair reagent concentrations are usually very low (approximately 0.02 M or less), a slight excess can reduce the selectivity substantially (Wangkarn and Pergantis, 2000). An

aqueous solution with an organic modifier is often used for elution and separation, *e.g.* methanol is usually used as the organic modifier in ICP-MS detectors to improve signal intensity and maintain plasma stability (for As and Se). Table 1.3 shows that both anion-pairing and cation-pairing chromatography were used for the separation of As species. Tetrabutylammonium (TBA, both hydroxide and phosphate) is the commonly used pairing cation for As species (As(III), As(V), MMA and DMA) (Martín et al., 1995; Pan et al., 2007). While hexanesulfonic acid (HSA) is often used in cation-pairing chromatography. The elution order of these species was consistently As(III), DMA, MMA and AS(V), independent of the various reverse-phase columns. In a wide pH range from 2 to 9, As(III) (pKa = 9.2) (Table 1.1) is a neutral species which eluted out in the void phase. Generally, the resolutions of these species are dependent of the concentration of ion-pair reagent, flow rate, ionic strength, and pH of eluent. H_2O was one of the most commonly used mobile phases for ion-pairing chromatography. Martín et al. (1995) developed a method for simultaneous speciation of As(III), AB, AC, DMA, MMA and As(V) using anion-pairing chromatography. $TBAPO_4$ was used as ion-pairing reagent, and H_2O as eluent. However, the result showed that AB and AC co-eluted. B'Hymer and Caruso (2007) speciated the same species using a cation-pairing chromatography with HSA as ion-pairing reagent. The mobile phase was prepared using citric acid (with a pH of 2.3) with methanol as modifier.

b) Ion-exchange chromatography

With ion-exchange chromatography, ions or easily ionized analytes of As, Sb and Se were separated, *e.g.* anion-exchange columns were used for separation of As(III), As(V), MMA, DMA, whereas cation exchange columns were used for separation of AB, AC, TMAO and Me_4As^+. Commonly, R_4N^+, SO_3^-, $RCOO^-$ were used as ion-exchanging groups (Weis and Weiss, 2004). Charge density and polarizability of the analytes depends on the molecule size and the charge (controlled by proton association-dissociation equilibrium). The pKa of As species occupy a large range, many of them being higher than 8 (Table 1.1). Hence, their negative charges are pH dependent. In addition, the protonation-deprotonation equilibrium of exchange sites, is also controlled by pH. Ion-exchange chromatography has been widely used for As inorganic species speciation, as the eluent pH can be better realized by AEX compared to other chromatography. CEX did not retain the two most toxic and most common species, As(III) and As(V), thus eluting them together in the front. Ponthieu et al. (2007) developed a method for As

inorganic and organic species speciation in landfill leachate using CEX on a PRP-X200 column. The results showed that As(III), MMA, As(V) and Cl^- eluted out in the front within 3 min, however, Arsenocholine (AC) and Trimethylarsineoxide (TMAO) were co-eluted at 15 min. Generally, anion-exchange chromatography can be used in a wide pH range (Table 2.1) and different eluents need to be chosen based on the existing form of As species and pH. HNO_3 was often used as mobile phase at low pH. Mattusch and Wennrich (1998) and Kohlmeyer et al. (2002) used an anion-exchange column with HNO_3 as mobile phase to analyze inorganic and organic As species. Based on this method, up to 17 As species were identified. For high pH above 9 (As existed as negatively charged $H_2AsO_4^-$), Hydroxide and carbonate containing eluents (NaOH, NH_4HCO_3 or $(NH_4)_2CO_3$) have widely been used on a variety of polymeric anion-exchange columns (Table 2.1). One of the advantages of this type of AEX is that high pH eluents substantially increase the dissociation of protonated As species and increase their affinity for anion exchanger.

However, at oxic/basic conditions the oxidation of As(III) to As(V) may occur fast (Jackson and Bertsch, 2001; Raab et al., 2004). Besides, separations at high pH can suffer from metals (Mg, Ca, Al, Mn, Fe, Cu, etc) precipitation as hydroxides inside columns and adsorb As species. Thus this method was suitable for NaOH extracted soil samples. An anion-exchange column (*e.g.* polymeric Hamilton PRP-X100 column) with medium pH seemed the optimum separation condition for As species. Phosphate-based mobile phases were widely used (Day et al., 2002; Pizarro et al., 2003). Similar to anion-pairing chromatography, co-elution of AB and As(III) may occur at neutral pH conditions. However, As(III) could be separated from AB when pH was higher than 9 or using tartaric acid as mobile phase, due to the formation of anionic As (Ackley et al., 1999). Despite the advantages of phosphate as eluent, e.g. playing an indispensable part in displacing As(V) from strong adsorbent sites, shortcomings are obvious: loading of phosphorous and sulfur can produce polymeric depositions on the cones and inside of ICP instrument, thus leading to drop in sensitivity due to clogging (Milstein et al., 2002). Organic mobile phases such as potassium hydrogenphthalate and tris(hydroxymethyl)aminomethane (TRIS) were also used as eluents (Woller et al., 1998; Milstein et al., 2002), though excessive loading of organic carbon can vary As intensities. In addition, NH_4NO_3 was also investigated as potential eluent due to its pH-flexibility (ranging from 2 to 9) and plasma compatibility.

c) Ion-exclusion chromatography

Ion-exclusion chromatography was also used to speciate weakly ionized or neutral As species. Strong anion- or cation-exchange resins were often used. In contrast to ion-exchange chromatography, charges on ion-exchange resin are the same as of weakly ionized species (Haddad and Jackson, 1990). That is, negatively charged analytes are separated on a cation-exchange resin, *e.g.* negatively charged As species are separated using resin containing anionic sulfonate functional groups, whereas positively charged analytes are separated via anion-exchange chromatography. The basic separation principle is, strong anions (*e.g.* inorganic As species) cannot penetrate into the occluded liquid phase due to the repelling by anionic functional groups on the resin, thus are not retained by the column. Weakly ionized analytes or neutral molecules of As (*e.g.* AB) penetrate the resin zone and move into the occluded liquid phase, thus result in different retention times. Up to 8 As species (As(III), As(V), MMA, DMA, AB, TMAsO, AC and TMAs) were determined using an ion-exclusion column packed with a carboxylated methacrylate resin and Na_2SO_4 as mobile phase (pH of 3.8), though an overall analysis time of over 60 min was used (Nakazato et al., 2000).

d) Other techniques

In order to increase the sensitivity of analytes, various nebulizers (ultrasonic nebulizer, thermospray nebulizer and so on) and hydride generation techniques were investigated. Among these, HG was favored, because it resulted in the highest sensitivity for As species, and eliminated clogging of samples and polyatomic ion spectral interferences of $^{40}Ar^{35}Cl$ on ^{75}As, as only gaseous species were introduced in plasma (Taniguchi et al., 1999).

Though for As speciation, AEX seemed the primary choice, a combination of an AEX (for separation of As(III), As(V), MMA and DMA) and a CEX column (for separation of AB, TMAO, AC and Me_4As^+) sometimes provides more information. This can be achieved using two columns in two procedures, or two columns in one procedure, *e.g.* dual column system (anion-exchange connected with cation-exchange) or dual mode system (a combination of ion-exclusion and cation-exchange) (Sakai et al., 2001).

Table 2.1 Speciation methods using HPLC for individual As, Sb and Se.

sample	analyte	column	eluent (pH)	detector	comment	Ref.
standard	As(III), AB, AC, DMA, MMA, As(V)	Ion pair (anion pairing)	H_2O (5.2)	HG-AAS	AB, AC coelute; $TBAPO_4$ as IP reagent	(Martín et al., 1995)
urine	AB, As(III), DMA, MMA, As(V)	Ion pair (anion pairing)	H_2O (5.8)	ICP-MS	TBAOH as IP reagent	(Pan et al., 2007)
apple extraction	As(V), As(III), MMA, DMA, AB, AC	Ion pair (cation pairing)	citric acid (2.3)	ICP-MS	HSA as IP reagent; MeOH as modifier	(B'Hymer and Caruso, 2007)
standard	As(III), As(V), DMA, AB, AC	Anion exchange	HNO_3 *Low pH*	ICP-MS	BDSA as modifier	(Mattusch and Wennrich, 1998)
fish, mussel, oyster and marine algae	As(III), As(V)… 17 As species	Anion exchange	HNO_3 *Low pH*	ICP-MS	BDSA as modifier; AB and Cl- coelute	(Kohlmeyer et al., 2002)
ground water	As(III), As(V), DMA, MMA, AB	Anion exchange	CO_3^{2-} (10.3) *High pH*	ICP-MS		(Larsen, 1998)
poultry waste	As(III), As(V), DMA, MMA, p-ASA, Rox	Anion exchange	NaOH (12.7) *High pH*	ICP-MS	MeOH as modifier	(Jackson and Bertsch, 2001)
urine, fish	As(III), As(V), DMA, MMA, AB	Anion exchange	$(NH_4)_2CO_3$ (9) *High pH*	DRC-ICP-MS	MeOH as modifier	(Wang et al., 2007)
water	As(III), As(V), DMA, MMA	Anion exchange	Na_3PO_4 (6) *Medium pH*	ICP-MS	EDTA as modifier	(Day et al., 2002)
food, sediment	As(III), As(V), DMA, MMA, AB	Anion exchange	$(NH_4)_3PO_4$ (6) *Medium pH*	ICP-MS	As(III) and AB co-elute	(Pizarro et al., 2003)

surface water	As(III), As(V), DMA, MMA, Se species	Anion exchange	NH_4NO_3 (8.7)	ICP-MS		(Martínez-Bravo et al., 2001)
landfill leachate	As(III), As(V), DMA, MMA, AB, TETRA, AC, TMAO	Cation exchange	NH_4NO_3 (2.5)	ICP-MS		(Ponthieu et al., 2007)
river water	Sb(III), Sb(V), $TMSbCl_2$	Anion exchange	A: diammonium tartrate (5.5) B: KOH (12)	HG-AFS	elution order: Sb(V), $TMSbCl_2$, Sb(III)	(Miravet et al., 2004)
coal fly ash	Sb(III), Sb(V)	Reversed phase	Sodium butanesulfonate+ TMAO+ Malonic acid+ Ammonium tartrate+ Methanol (3)	ICP-MS	Simultaneous separation of As and Se redox couples	(Narukawa et al., 2005)
coal fly ash	Sb(III), Sb(V)	Anion exchange	A: diammonium tartrate (5.5) B: KOH (12)	ICP-MS		(Miravet et al., 2006)
soil	Sb(III), Sb(V)	Anion exchange	EDTA+ phthalic acid (4.5)	ICP-MS		(Amereih et al., 2005)
river water, soil	Sb(III), Sb(V), TMSbO	Anion exchange	phthalic acid (5.0) or 4-hydroxybenzoic acid (5.5)	ICP-MS, ICP-AES	elution order: Sb(V), TMSbO, Sb(III)	(Ulrich, 1998)
synthetic solutions	Sb(III), Sb(V), $TMSbCl_2$	Anion exchange	ammonium tartrate (pH gradient 2.3-1.5, 20 °C)	ICP-MS	elution order: Sb(V), Sb(III), $TMSbCl_2$,	(Nash et al., 2006)
synthetic solutions	Sb(III), Sb(V), $TMSbCl_2$	Anion exchange (weak)	ammonium tartrate (pH gradient 3.0-2.0, 60 °C)	ICP-MS	at 20 °C a system peak co-elute; at 60 °C no co-elute of system peak, but elution order reversed	(Nash et al., 2006)
plants	Sb(III), Sb(V), $TMSbCl_2$	Anion exchange	A: EDTA (4.5) B: EDTA + NH_4OH (11)	ICP-MS		(Müller et al., 2009)

yeast	20 Se compounds	Ion-pair reversed phase	methanol + H_2O (2.5)	ICP-MS ESI-MS	perfluorinated carboxylic acids as IP reagents. *e.g.* TFA, PFPA, HFBA(best). Overall time of 70 min	(Kotrebai et al., 2000)
soil	Se(IV), Se(VI), Se-cysteine SeMet	Anion exchange	salicylic acid-sodium salicylate	ICP-MS	elution order: SeMet, Se-cysteine, Se(IV), Se(VI)	(Ponce de León et al., 2003)
		Ion-pair reversed phase	water + methanol		HFBA as IP reagent elution order: Se(VI), Se(IV), Se-cysteine, SeMet	
human urine	Se(IV), Se(VI), SeMet, TMSe	Cation exchange	Oxalic acid + K_2SO_4 + methanol (3)	ICP-MS	elutin order: Se(IV), Se(VI), TMSe, SeMet	(Gammelgaard et al., 2001)
			Ammonium formate + methanol (3)		elution order: Se(IV), SeMet, TMSe. Se(VI) eluted last with broad peak	
rats blood cell, liver,	Se(VI), MMSe* TMSe	Size exclusion	Tris–HCl (7.4)	ICP-MS		(Shiobara et al., 1999)
human urine	SeMet, Selenosugars	Reversed phase	Ammonium formate +MeOH (3)	ICP-MS	Reversed phase chromatography was superior	(Kuehnelt et al., 2005)
		Anion exchange	Citric acid (4.8)			
		Cation exchange	Pyridine (1.6)			
Plants, (Indian mustard)	Se(IV), Se(VI), SeCys, Se-MSeCys, S-(MSe)Cys, Se-Met-Se-oxide hydrate	Ion-pair reversed phase	water + MeOH		perfluorinated carboxylic acids as IP reagents. *e.g.* TFA, PFPA, HFBA (best), NFPA; resolution increase with longer chain-length of IP reagents	(Kahakachchi et al., 2004)
human serum	Selenoproteins (*e.g.* glutathione peroxidase, selenoprotein P and albumin	affinity	A: Tris-HCl (7.4) B: Tris-HCl + ammonium acetate (7.4)	ORS-ICP-MS	post-column isotope dilution methodology was used	(Hinojosa Reyes et al., 2003)
		anion exchange	A: Tris-HCl (7.4) B: Tris-HCl + ammonium acetate (7.4)		separation not satisfactory	

biological tissues	Se(IV), Se(VI), SeMet SeCys TMSe	cation exchange	pyridine solution (2.8 and 4.7)	ICP-MS	elution order depend on pH	(Moreno et al., 2004)
		size exclusion	phosphate buffer in NaCl (7.2)			
water sample	Se(IV), Se(VI), SeMet SeCys	reversed phase	phosphate (6)	ICP-MS	Se(IV), Se(VI) showed anion exchange mechanism; SeCys showed reversed phase mechanism	(Quijano et al., 1996)

TFA: trifluoroacetic acid; **PFPA:** pentafluoropropanoic acid; **HFBA:** heptafluorobutanoic acid Sb speciation
SeMet: selenomethionine; **TMSe:** trimethylselenonium; **SeCys:** Se-cysteine
MMSe*: MMSe related Se compound; **ORS:** octapole reaction system
BPDTC: dithiocarbamate, benzylpropionitrile dithiocarbamate

2.2.2 Sb speciation

Compared to As speciation analysis, the separation of Sb species was more difficult. Chromatographic techniques were applied less for speciation analysis of Sb species, especially for Sb organic species. The possible reason might be the lack of commercial standards for organic Sb compounds, making it impossible to quantify these compounds accurately. Sb(III), Sb(V) and TMSb are three main Sb species that were widely determined using chromatography methodology.

a) Anion-exchange chromatography

Sb anionic species predominate in aqueous environmental matrices. Therefore strong anion-exchange chromatography (Hamilton PRP-X100, Dionex AS4A, or ION-120) was widely used for Sb speciation. Under these conditions, Sb(V) was readily eluted, while Sb(III) was strongly retained on the column, which was indicated by long retention time and severe peak tailing. To solve these problems, different studies were made including using a shorter guard column, however, broad peak for Sb(III) was still observed. Another way was using of complexing mobile phases, *e.g.* (di)ammonium tartrate (Miravet et al., 2004; Miravet et al., 2006; Nash et al., 2006), EDTA (Amereih et al., 2005; Müller et al., 2009), 4-hydroxybenzoic acid (Ulrich, 1998) and phthalic acid (Ulrich, 1998; Amereih et al., 2005), though one drawback of this technique was that Sb(V) was normally eluted close to or even in solvent front. This cannot facilitate speciation of Sb species. Cation and reversed-phase columns were investigated, too (Lintschinger et al., 1997). However, basically no obvious improvements were obtained compared to anion-exchange column. In addition, pretreatment of samples was used to solve Sb(III) tailing problems, *e.g.* by adding certain organic ligands to samples, Sb(III) could be chelated and formed stable complexes, which facilitated further separation on a chromatographic column. A certain number of organic compounds (EDTA, DTPA, CDTA, BPDTC) (Kolbe et al., 2012; Park and Hardy, 1989; Er-kang, 1982) were investigated as potential complexation ligands for Sb(III). However, significant studies have shown that stable Sb(III)-complexations were observed only at acidic pH ranges. These ligands do not prevent Sb(III) hydrolysis at pH > 6.

The organic Sb species of TMSb can be easily separated from Sb(V) on anion-exchange chromatography with phosphate, carbonate, or potassium as mobile phase. However, it

always eluted out in solvent front, which is not desirable for identification of Sb species. Besides, previous study found that $TMSbCl_2$ was only eluted under alkaline conditions in solvent front on reversed phase chromatography, and even not detectable on cation-exchange chromatography (Lintschinger et al., 1997). This might be explained by the polymerization or condensation phenomenon of $TMSbCl_2$ under neutral and acidic conditions. Table 1.3 shows that if Sb(III), Sb(V) and TMSb are analyzed simultaneously, an eluent gradient were normally involved; one with organic compounds at acidic pH to chelate and elute Sb(III), the other with simple inorganic solution (KOH or NH_4OH) at alkaline pH (higher than 10) in order to elute TMSb at non-solvent front retention time (EDTA or diammonium tartrate) (Müller et al., 2009; Miravet et al., 2006). Besides, pH gradient was also investigated (Nash et al., 2006).

b) Reversed phase chromatography

Though reversed phase chromatography proved to be ineffective for TMSb analysis, it could still be used for inorganic Sb species speciation. Narukawa et al. (2005) developed a method for simultaneous speciation of As, Sb and Se inorganic species using reversed phase chromatography (CAPCELL PAK C18 MG S5 ODS column) with sodium butanesulfonate/tetramethylammonium hydroxide/malonic acid/ammonium tartrate/methanol (pH 3.0) as mobile phases. ^{78}Se was monitored on an ICP-MS 7500c (Agilent, Japan) with collision reaction cell (He as reaction gas).

2.2.3 Se speciation

Compared to As and Sb speciation, Se can form more organic compounds such as Se proteins, and a larger choice of chromatography is available for Se species speciation analysis, *e.g.* ion-pair reversed phase chromatography, size exclusion chromatography, anion-exchange chromatography, cation-exchange chromatography, affinity chromatography. Generally, for Se inorganic species, anion-exchange chromatography with common mobile phases (ammonium, formate or phosphate) is sufficient. For Se organic compounds such as SeMet, TMSe and Se-cysteine, ion-pair reversed phase chromatography was widely used. Size exclusion and affinity chromatography, however, were normally used for Se proteins speciation.

a) Reversed phase chromatography

This kind of chromatography facilitates the separation of Se ionic species, as well as uncharged molecular species. As a counter ion (from ion-pair reagents) was often added to mobile phase, a secondary chemical equilibrium was introduced to control selectivity and resolution of the analytes. Separation of analytes by ion-pair HPLC is influenced by different variables, including buffer concentration, pH and ionic strength of mobile phase, hydrophobicity of counter ion, as well as properties of stationary phase. As for As species, long-chain alky ions were often used as ion-pair reagents. Perfluorinated carboxylic acids such as TFA, PFPA, HFBA and NFPA were investigated as ion-pair reagents on an ion-pair reversed phase column for speciation of Se species from yeast samples (Kotrebai et al., 2000). The result showed that HFBA performed best and allowed speciation of up to 20 Se compounds, though an overall analysis time of 70 min was needed. In another study (Kahakachchi et al., 2004), these perfluorinated carboxylic acids were also tested. Apart from previously reported organoselenium species such as SeMet and Se-methylselenocysteine, it also allowed speciation of S-(methylseleno)cysteine and Selenomethionine Se-oxide hydrate. This suggests that resolution of Se species increases with longer chain-length of ion pair reagents. Similar to As, reversed phase chromatography used for separating Se species also normally utilized water as mobile phase and methanol as modifier. Kuehnelt et al. (2005) separated and determined 3 selenosugars (selenium metabolites), namely methyl-2-acetamido-2-deoxy-1-seleno-β-D-galacto-pyranoside, methyl-2-amino-2-deoxy-1-seleno-β-D-galactopyranoside and methyl-2-acetamido-2-deoxy-1-seleno-β-D-glucosopyranoside using reversed phase chromatography with ammonium formate as mobile phase. In this kind of chromatography SeCys and SeMet exist as zwitterions, whereas selenite and selenate occur in anionic form.

b) Size exclusion chromatography

Size exclusion chromatography (SEC) is suitable for extraction and separation of Se soluble protein fractions (Moreno et al., 2004; Shiobara et al., 1999). In general, the separation of different fractions on SEC column depends on relative size of analytes and pores on porous stationary phase. Unlike other chromatography, small compounds of similar molecular mass, may be eluted in addition to selenoproteins (Behne et al., 1998). The results from SEC-ICP-MS or SEC-UV provide useful information on further purification and amino acid sequence determination of Se protein, which can help researchers better understand the specific function of Se in biochemical processes of

various organisms. Thus SEC was often used in combination with other chromatography, such as cation-exchange chromatography (Moreno et al., 2004).

c) Ion-exchange chromatography

Both anion- and cation-exchange chromatography were used for separation of Se ions and easily ionized Se analytes. As has been mentioned, variables, such as, ionic strength of solute and mobile phase, pH of mobile phase, can influence the elution and resolution of analytes. Although common eluents of phosphate or ammonium were often used for inorganic Se species separation, complex organic mobile phases were necessary if organic Se species such as SeMet, Se-cysteine were to be separated. Ponce de León et al. (2003) separated four Se species: Se(IV), Se(VI), Se-cysteine and SeMet using anion-exchange chromatography with salicylic acid - sodium salicylate as mobile phase. In addition, oxalic acid (Gammelgaard et al., 2001), Tris-HCl (Hinojosa Reyes et al., 2003), citric acid and pyridine (Kuehnelt et al., 2005) were investigated as potential mobile phases.

The cation-exchange column possesses some anion-exchange properties, which also allow anion separation (Gammelgaard et al., 2001; Moreno et al., 2004). Table 2.1 shows that cation-exchange chromatography was mainly used for $TMSe^+$ speciation. Pyridine solution was often used as mobile phase. Matrix influence also played an important part in separating Se species. *E.g.* Se speciation in urine is still problematic and the results of different studies were even contradictory. Besides, some studies showed distortion of signal intensity by varying and high concentrations of salt when urine samples were chromatographically separated without pretreatment (Gammelgaard et al., 2001).

d) Other chromatography

Hinojosa Reyes et al. (2003) developed a method for Se-containing proteins speciation analysis in human serum using affinity chromatography coupled to ICP-MS. A post-column isotope dilution analysis (IDA) methodology was applied for quantification and an octapole reaction system (ORS) was used to eliminate argon interference on ^{78}Se and ^{80}Se. The method was further validated using an anion-exchange chromatography. The results showed that affinity chromatography performed better than anion-exchange chromatography and finally three Se fractions; selenoprotein P, albumin and glutathione

peroxidase were confirmed and determined. Quijano et al. (1996) developed a method for simultaneous speciation of Se(IV), Se(VI), SeCys and SeMet using a mix column (Spherisorb ODS-AMINO), where the stationary phase consisting of an equimolar mixture of octadecyl and amino groups on a silica support, acting as a reversed-phase, weak anion-exchange or ion-pair chromatography. Phosphate buffer at pH of 6.0 was used as mobile phase. The elution order of analyzed Se species (SeCys, SeMet, Se(IV) and Se(VI)) showed that Se(IV) and Se(VI) followed an anion-exchange chromatography mechanism, while SeCys and SeMet (both existed as zwitterions in the pH range of 2.0 to 8.0) indicated a reversed phase chromatography (though SeCys contained terminal polar and ionized function, it eluted out earlier than SeMet).

2.3 Gas chromatographic separation

2.3.1 As speciation

Headspace injection, solid phase micro-extraction (SPME), cryotrapping and chemotrapping were often coupled with GC-MS for speciation of volatile As species, such as AsH_3, $MeAsH_2$, Me_2AsH and Me_3As. However, direct headspace injection may cause too high detection limits which are not sufficiently sensitive for real sample determination. SPME (PDMS, PDMS-CAR and PDMS-CAR-DVB are often used as SPME fibre) (Planer-Friedrich et al., 2006; Kösters et al., 2003; Mester et al., 2001), cryotrapping (Krupp et al., 2007, 2008; Yuan et al., 2010) and chemotrapping (Uroic et al., 2009) are more practically used. Kösters et al. (2003) analyzed environmental compost samples (samples were first derivatized with $NaBH_4$ and speciation using GC-ICP-MS) as well as hydrothermal vapor phase (gas was sampled with SPME). The results showed that seven As species were identified existing in environment, and three of them (diarsine, monomethyl diarsine and dimethylarsenomercaptane) were reported in environmental samples for the first time. In another work, SPME-GC-ICP-MS was applied to analyze volatile As species in the geothermal systems of Yellowstone National Park. The vapor phase of hydrothermal systems has been found playing an important part in transporting As species (0.5–200 mg m^{-3} of volatile As species present in vapor phases (Planer-Friedrich, 2004). The results showed that $(CH_3)_2AsCl$, not $(CH_3)_3As$ as expected from previous studies, was the most frequently detected volatile As species. Seemingly, higher concentration of $(CH_3)_3As$ was determined when As(III) was dominant in aqueous phase. Generally, volatile As species of $(CH_3)_2AsCl$, $(CH_3)_3As$, $(CH_3)_2AsSCH_3$ and CH_3AsCl_2 were detected in decreasing order of frequency.

Compared to cryotrapping, chemotrapping is more suitable for field study and sampling. Uroic et al. (2009) developed a method for quantitative chemotrapping of $(CH_3)_3As$ using silver nitrate impregnated silica gel filled tubes. Natural gas samples were analyzed and UV-HG-AFS was used for determination. The developed method was also validated using CT-GC-ICP-MS as a reference method. Yuan et al. (2008) developed an online collection and speciation device for volatile As species produced by bacteria by combining a bacterial incubation and CT-GC-ICP-MS. He gas was used for purging analytes. The researchers further improved the device by replacing GC column with a packed cotton column which was immersed into liquid nitrogen (Yuan et al., 2010). Detection was achieved on an AFS detector. This new device avoided potential error resulted from pressure changes of the ICP system. Because purging, followed by trapping, may cause a sudden increase of system pressure, thus leading to gaseous arsenicals leakage and plasma shutdown.

2.3.2 Sb speciation

Volatile Sb compounds such as stibine (boiling point (BP) –17 °C), methylstibine (BP 41 °C), dimethylstibine (BP 61 °C) and trimethylstibine (BP 81 °C) can easily be separated with a GC column. AAS, AFS or ICP-MS have been used for determination. Sampling strategies of headspace injection, solid phase micro-extraction (SPME), cryotrapping (or low temperature trapping) were widely used. Krupp et al. (1996) developed a method for identifying and quantifying several volatile metal and metalloid compounds, *e.g.* alkylated As and Sb species and methylated Se species. Samples were first derivatized by HG (with $NaBH_4$), then cryofocused in a trap (a U-shape column packed with Supelcoport (10% SP-2100)). Determination was carried out on an ICP-MS. In another work, Hirner et al. (1998) applied GC-ICP-MS for metal(loid)organic species (Hg, As, Sb…) in geothermal gases and waters. Gases were collected using a Tedlar bag, and cryogenically pre-concentrated on Chromosorb (10% SP-2100) at -78 °C (dry ice) and cryo-focused on a second trap at -196 °C (liquid N_2). For aqueous samples, derivatization by HG was used to form gaseous hydrides. Volatile species of As, Sb, Hg and Se species (*e.g.* AsH_3, $AsMeH_2$, $AsMe_2H$, $AsMe_3$, $SbMe_3$ and $SeMe_2$) could be detected in gases over hot springs, and Me_3Sb was the only methylated Sb compound in gases of geothermal waters. The lack of pure standards or reference samples is also problematic for volatile Sb compounds speciation. The trimethylantimony, such as

$(CH_3)_3SbCl_2$ has been widely used as a standard compound for identifying volatile Sb species, and based on that some researchers have detected mono- and di-methylantimony species from natural water using HG technique (Andreae et al., 1981). However, in some recent studies concerning derivatization of $(CH_3)_3SbCl_2$ using HG (Dodd et al., 1992; Dodd et al., 1996), several other products such as $(CH_3)_3Sb$, $(CH_3)_2SbH$, CH_3SbH_2 and SbH_3 were also detected. This means that previous detection of some volatile Sb species are now doubtful, because they may be derived from $(CH_3)_3SbCl_2$. Craig et al. (1999) minimized the byproducts by rigorous exclusion of oxygen and rapid purging of reduced analytes into a cold trap when using trimethylantimony as a standard to detect methylantimony with HG-CT-AAS. Duester et al. (2005) identified and quantified volatile As and Sb species in depth profiles of various soil samples by using pH-gradient HG coupled with purge-and-trap-GC-ICP-MS. During derivatization with $NaBH_4$, pH was initially adjusted to 7 (with citrate buffer) and gradually decreased to 1. The molecular information of the detected species was obtained by parallel ESI-MS and ICP-MS detection. In addition, solid phase micro-extraction (SPME) was also used for volatile Sb species speciation (Smith et al., 2002).

2.3.3 Se speciation

The technique of GC has long been successfully used for organic Se species speciation, due to its high sample transport efficiency and high sensitivity compared to CE and HPLC. Organic Se species were often found in environment, which could be generated by bacteria and micro-organisms. The common volatile organic Se species are: hydrogenselenide, dimethylselenide, dimethyldiselenide, dimethylseleniumsulfid and dimethylseleniumdioxide. Evans and Johnson (1966) separated dimethyl, diethyl and di-*n*-propyl selenides; dimethyl, diethyl and di-*n*-propyl diselenides; ethyl selenocyanate with a polymetaphenylether column using GC technique. A trapping system plays an important part for volatile Se species speciation using GC. Traditional strategies like Headspace SPME were widely used (Gabel-Jensen et al., 2010; Diaz-Bone and van de Wiele, 2009; Sanz Landaluze et al., 2004; Meija et al., 2003; Meija et al., 2002). However, they normally do not allow simultaneous separation and determination of several species of different elements. Pécheyran et al. (1998) developed an automated filed cryotrapping device to collect air samples at -175 °C. Samples then could be flash-desorbed in the lab in a cryogenic trapping-gas chromatography- ICP MS (CT-GC-ICP-MS) for determination of volatile species. Based on this method, Amouroux et al. (1998)

developed an in-situ purge and cryogenic trapping method for pre-concentration of volatile species in natural water samples followed by gas chromatography and inductively coupled plasma mass spectrometry (P-CT-GC-ICP-MS). Recently, a simple and portable chemotrapping technique was developed for volatile methylated selenium species with nitric acid (Winkel et al., 2010). Quantitative recovery of 65.2%±1.9% for $(Me)_2Se$ and 81.3%±3.9% for $(Me)_2Se_2$ could be achieved.

In addition, the non-volatile Se species (*e.g.* Selenomethionine (SeMet)) can also be analyzed using GC-ICP-MS after derivatization of the analytes. Yang et al. (2004) determined the SeMet in yeast using GC-ICP-MS flowing digestion with methanesulfonic acid and derivatization with cyanogen bromide (CNBr). Vonderheide et al. (2002) analyzed seleno amino acids, selenomethionine (SeMet), selenoethionine (SeEt) and selenocystine (SeCys) using solid-phase micro-extraction (SPME) as preconcentration strategy followed by GC-ICP-MS. Isobutylchloroformate was used to increase the species volatility (acylation of the amino group and esterification of the carboxylic group).

Table 2.2 Speciation methods using GC for individual As, Sb and Se.

Sample	Analyte	Sampling	Separation & Detection	References
hot spring vapor phase	Me_2AsCl, Me_3As, Me_2AsSMe, $MeAsCl_2$	SPME	GC-MS	(Planer-Friedrich et al., 2006)
environmental compost	As_2H_4, $MeAs_2H_3$, Me_2AsSMe. et al.	SPME	GC-MS	(Kösters et al., 2003)
natural gases	Me_3As	chemotrapping	UV-HG-AFS	(Uroic et al., 2009)
Bacterial incubation	AsH_3, $MeAsH_2$, Me_2AsH, Me_3As	cryotrapping	HG-AFS	(Yuan et al., 2010)
sediment of river and harbour	$SbMeH_2$, $SbMe_2H$, $SbMe_3$, $SbEt_3$	HG and cryotrapping	HG-CT-GC-ICP-MS	(Krupp et al., 1996)
hot spring water and gases	Me_3Sb	HG and cryotrapping	HG-CT-GC-ICP	(Hirner et al., 1998)
plants	Me_2SbH	HG and cryotrapping	HG-CT-AAS	(Craig et al., 1999)
soil	$SbMeH_2$, $SbMe_2H$, $SbMe_3$	pH gradient HG-PT	GC-EI/MS-ICP-MS	(Duester et al., 2005)
in vitro metabolism	MeSeH, Me_2Se and Se_2 $(Me)_2$	Headspace	GC-MS	(Gabel-Jensen et al., 2010)
intestinal microorganisms	MeSeH, Me_2 Se and Se_2 $(Me)_2$	Headspace	GC-ICP-MS	(Diaz-Bone and van de Wiele, 2009)
production and gastric digestion processes of selenized yeast	Me_2Se, $Se_2(Me)_2$	SPME	GC-MIP-OES	(Sanz Landaluze et al., 2004)
roasted coffee	MeSeH, Me_2Se, $Se_2(Me)_2$, MeSeSEt, MeSeSMe, MeSeSeEt, $Se_2(Et)_2$, Et_2Se	SPME	GC-ICP-MS	(Meija et al., 2003)
Se accumulating plants	Me_2Se, $Se_2(Me)_2$, MeSeSMe	SPME	GC-ICP-MS	(Meija et al., 2002)
air samples in urban, rural and industrial environments	Me_2Se and other volatile metal and nonmetal species (*e.g.* Hg, As)	cryotrapping	CT-GC-ICP-MS	(Pécheyran et al., 1998)
natural water	Me_2Se, Me_2Se_2 and other volatile metal species (*e.g.* Hg, Sn)	in situ purge and cryogenic trapping	CT-GC-ICP-MS	(Amouroux et al., 1998)
yeast	SeMet	Derivatization and solvent extraction	GC-ICP-MS	(Yang et al., 2004)
standards	seleno amino acids, SeMet, SeEt, SeCys	SPME	GC-ICP-MS	(Vonderheide et al., 2002)

MIP: microwave induced plasma OES: optical emission spectrometry PT: purge and trap EI: electron ionization

3. Scopes and objectives

The main objective of the present study is development and optimization of methods for simultaneous speciation analysis of inorganic As, Sb and Se species, and application of the developed method to the analysis of real fluid samples.

To achieve these goals the project involves:

- Development of a chromatographic method for simultaneous separation of As(III) and As(V), Sb(III) and Sb(V), and Se(IV) and Se(VI) based on HPLC coupled to ICP-MS.
- Stability study of As, Sb and Se redox couples in Fe- and Mn-rich water samples, with the purpose of finding a potential preservation strategies (light, temperature, acidification) for these species in a long time scale (*e.g.* 3 months).
- Application of the developed method to analysis of hydrothermal waters (with hydrothermal samples from Bali and Java, Indonesia).

To address these questions, the following studies were carried out within this dissertation:

In chapter 4 “Simultaneous speciation analysis of As, Sb and Se redox couples by SF-ICP-MS coupled to HPLC”. A new method was developed for the simultaneous speciation analysis of inorganic As(III, V), Sb(III, V) and Se(IV, VI) in fluid samples by SF-ICP-MS coupled with HPLC. Up to date only scare reports can be found in literature concerning simultaneous speciation of these species. The key factor in this study is finding the appropriate eluent. Because the adequate mobile phase composition for each element is different, *e.g.* in most cases Sb(III) has a very strong affinity towards stationary phases and would be irreversibly retained on some columns without chelators. In this work a Hamilton PRX-X100 anion-exchange column with EDTA (pH of 4.7) and 3% methanol as mobile phase was used for separation of the six desired species. The overall analysis time was less than 11minutes for all species with a solvent gradient (linear ramp from 5 mM to 30 mM) being introduced in.

In chapter 5 “Preservation and stability of As, Sb and Se redox couples in water samples”. The simultaneous preservation of the following redox couples was studied: As(III, V), Sb(III, V) and Se(IV, VI). Successful preservation of these species without any inter-conversion was the prerequisite for accurate analysis of the distributions of these species in environment. However, to our knowledge, a preservation strategy for inorganic species of As(III, V), Sb(III, V) and Se(IV, VI) simultaneously has not yet been reported. There are even discrepancies regarding preservation of individual As, Sb or Se species. For example, acidification is one of the most common procedures for preserving As species. However, some researcher argue that it may cause an immediate oxidation of As(III) to As(V) with addition of HCl or HNO_3. In this work, EDTA at low pH (around 3) was studied as potential preservation methodology over a time period of 11 weeks in Fe- and Mn-rich water samples. The results showed that addition of EDTA combined with acidification to a pH of 3 successfully preserved all three redox couples when stored at 4 °C in the dark. In addition, the oxidation and adsorption behavior of these species with the presence of Fe-(oxy)hydroxide were also studied.

In chapter 6 “As and Sb redox species in hydrothermal waters from Bali and Java, Indonesia”. The developed method was successfully applied to analysis of hydrothermal fluids. With the samples from Bali and Java, Indonesia, the distribution of As and Sb species was studies. The relationship of the existing form and Cl^-, HCO_3^- and SO_4^{2-} concentration was studied. To date, speciation of As and Sb inorganic species was still scarcely reported, probably due to the lack of simultaneous speciation method and inability of preserving the distribution of these species as identical as in original environment. Field separation method using a column may be suitable for two or three analytes with enough resolution, but not applicable for more than four analytes. Besides, it is unable to isolate unknown species. For example, in this work an unidentified species was detected, which was even the dominant As species in two of the samples. This provided useful information on predicting other involved oxidation processes, like microbial activity.

The following list gives my contributions to each chapter:

Chapter 4: “Simultaneous speciation analysis of As, Sb and Se redox couples by SF-ICP-MS coupled to HPLC”

- Literature study
- Experiment designing
- Experimenting with HR-ICP-MS coupled to HPLC from method development to validation
- Dealing with data
- Manuscript writing and editing

Chapter 5: "Preservation and stability of As, Sb and Se redox couples in water samples"

- Literature study
- Experiment designing
- Sample collection and preservation
- Measuring of samples with HR-ICP-MS coupled to HPLC in a time scale of 11 weeks
- Dealing with data
- Manuscript writing and editing

Chapter 6: "As and Sb redox species in hydrothermal waters from Bali and Java, Indonesia"

- Literature study
- Measuring of samples for As and Sb species using HR-ICP-MS coupled to HPLC
- Dealing with data
- Manuscript writing

4. Simultaneous speciation analysis of As, Sb and Se redox couples by SF-ICP-MS coupled to HPLC

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Abstract

A new method was developed for the simultaneous speciation analysis of inorganic arsenic (III, V), antimony (III, V) and selenium (IV, VI) in fluid samples by double-focusing sector field-inductively coupled plasma-mass spectrometry (SF-ICP-MS) coupled with high performance liquid chromatography (HPLC). A Hamilton PRX-X100 anion-exchange column with EDTA (pH of 4.7) and 3% methanol as mobile phase was used for separation of the six species. The flow rate was set at 1.5 mL min^{-1}. The overall analysis time was shortened down to within 11minutes for all six desired species after a solvent gradient (linear ramp from 5 mM to 30 mM) was introduced in. The detection limits for As(III), As(V), Sb(III), Sb(V), Se(VI) and Se(IV) were 0.02 µg L^{-1}, 0.06 µg L^{-1}, 0.2 µg L^{-1}, 0.02 µg L^{-1}, 0.2 µg L^{-1} and 0.4 µg L^{-1} respectively, which were obtained from 11 replicate measurements of blank. The stability of retention time and linearity of calibration curve were also evaluated. Relative standard deviations (RSD) of ≤ 9% for retention times (at least 20 replicate measurements) and correlation coefficients (R^2) of ≥ 0.9998 for calibration curves (at least 6 replicate experiments) were obtained. Finally, the proposed method was applied to the analysis of one synthetic sample, two hot spring samples and two certified reference materials. The results showed a good spike recovery, indicating that basically no mass loss occurred during chromatographic separation. For two certified reference materials, the detected results were in good agreement with the certified values.

4.1 Introduction

Despite our effort to better understand the geochemistry of redox sensitive, multi species elements, such as, arsenic (As), antimony (Sb), chromium (Cr), cobalt (Co), copper (Cu) and selenium (Se), there are still large gaps in our knowledge, particularly with respect to their redox behavior in different environments. It has been realized that a full understanding of the redox behavior of these species can help us to: (a) better understand the redox behavior of these elements in different matrices (Breuer and Pichler, 2013; Vodyanitskii, Yu, 2010; Leuz, 2006); (b) develop further studies in the area of toxicity and bioavailability, for example, different toxicity and bioavailability as a function of redox state (Price et al., 2013; Mailloux et al., 2009; Price et al., 2007; Jamier et al., 2010); (c) evaluate competitive adsorption of, for example, arsenic and antimony, onto hydrous ferric oxide (HFO) surfaces, which in turn would let us better predict their mobility (Nakamaru and Sekine, 2008; Campbell et al., 2006; Wilson et al., 2010). However, conventional total element concentration determination did not provide adequate information to completely understand the effect, behavior and fate of these redox species in the environment. In view of this hyphenated analytical techniques such as HPLC-HG-AFS, ETV-ICP-MS (Li et al., 2008) and HPLC-ICP-MS (Moldovan et al., 2004) were developed to obtain additional information about the distribution of individual redox species. Since the distribution of redox species of a given element in an aqueous solution greatly depends on the species distribution in other redox couples an accurate and rapid simultaneous speciation analysis method for multiple redox couples would be the logical next step towards an improved understanding of redox chemistry in natural systems.

Redox speciation analysis requires analytical technology, which basically includes species separation followed by detection, where due to often low concentrations and minute sample amounts detector can be the weak link. If it is desired to speciate more than one redox couple chromatographic separation becomes critical and difficult, because the chromatography conditions for different elements can vary substantially. For example, inorganic As species are usually separated with anion-exchange chromatography with phosphate buffers at neutral pH as mobile phase (Zheng et al., 2003; Day et al., 2002) and anion-exchange column such as Hamilton PRP-X100 column is most commonly used (Milstein et al., 2002; Bednar et al., 2004). Se species are commonly separated with anion-exchange chromatography as well, with phosphate,

ammonium or citrate buffers as mobile phase (Guerin et al., 1997). However, Sb behaves quite differently, as it has complexing properties and needs the presence of a chelating agent in the mobile phase. EDTA (Miekeley et al., 2002; Canepari et al., 2010; Krachler et al., 2001) and phthalic acid (Smichowski et al., 1998; Hansen and Pergantis, 2007; Zheng et al., 2001; Ceriotti and Amarasiriwardena, 2009) have been widely used as mobile phase. In the wide pH range from 2 to 10 in aqueous solutions, Sb(III) exists in the form of non-charged $Sb(OH)_3°$ in liquid, while Sb(V) exists as negatively charged $Sb(OH)_6^-$ (Takayanagi and Cossa, 1997). Theoretically, $Sb(OH)_3°$ and $Sb(OH)_6^-$ can be separated using an anion-exchange column, then $Sb(OH)_3°$ would be eluted in solvent-front, and $Sb(OH)_6^-$ would be retained in the column and eluted out subsequently. However, the fact is that $Sb(OH)_6^-$ elutes close to the solvent front and $Sb(OH)_3°$ is strongly retained in the column and can not be eluted with common mobile phases similar to those used for As and Se speciation. This provides a challenging task in finding the optimum mobile phase, which would allow the separation of As, Se and Sb redox couples. A few methods for the simultaneous speciation analysis of As, Sb and Se are listed in Table 4.1. Only one of the methods, however, allowed complete speciation analysis of the three redox couples, and was only optimized with synthetic standards (Lindemann et al., 1999). Guerin et al. (1997) developed a speciation analysis method for As(III, V), Se(IV, VI) and Sb(V), but Sb(III) was not included. Orero Iserte et al. (2004) and Morita et al. (2007) only considered the speciation analysis of two of the three elements. Regarding the stationary phases, anion-exchange was the typical choice for the simultaneous separation of those species. However, Morita et al. (2007) used a reversed phase chromatographic column, basically because they were interested in the separation of other organic As species. For the separation of As and Se species, phosphate and hydrogen carbonate buffers are adequate, when anion-exchange chromatography is used. If Sb should be separated together with Se and As species, a chelating agent, such as tartaric acid, malonic acid or EDTA is usually added.

Following a successful chromatographic separation of the three redox couples, simultaneous detection and thus speciation analysis still requires sensitive detection. ICP-AES, ICP-AAS and AFS were extensively investigated but not practically applied, because of low sensitivity and spectral interferences specially for Se (Uden, 2002; Dauchy et al., 1994; B'Hymer and Caruso, 2006; Bowman et al., 1997). Thus an extra analytical step, such as hydride generation (HG), was used to improve its sensitivity

(Cabon and Louis Madec, 2004; Wu et al., 2011; Fernandez et al., 1992). However, HG-ICP-AES, HG-ICP-AAS, HG-AFS are not suitable for direct Se(VI) determination, due to its inability to form a Se-hydride (Dauchy et al., 1994; Ipolyi and Fodor, 2000; Kozak and Niedzielski, 2011; Richter et al., 1998).

In this paper we presented a new method for the simultaneous speciation analysis of As(III, V), Sb(III, V), and Se(IV, VI) using double-focusing sector field-inductively coupled plasma-mass spectrometry (SF-ICP-MS) coupled to high performance liquid chromatography (HPLC). In addition to low detection limits and good separation one of the goals was to keep the chromatographic conditions as simple as possible, with the idea that this should facilitate implementation of this method by others interested in the redox behavior of natural aqueous solutions.

4.2 Experimental

4.2.1 Instrumentation

4.2.1.1 Detection

A Thermo Scientific ELEMENT 2 sector field ICP-MS (SF-ICP-MS) was used for the detection of As(III, V), Sb(III, V), and Se(IV, VI). The instrumental conditions and tuning information are given in Table 4.2. The inlet system consisted of a Scott type double pass spray chamber (G.E.) and a Conikal nebulizer (G.E.), whose uptake rate was compatible with the flow rate of the HPLC (1.5 mL min^{-1}). For As and Sb, isotopes of ^{75}As and ^{121}Sb were monitored. For Se, the less abundant isotope of ^{78}Se or ^{82}Se had to be used due to the $^{40}Ar^{40}Ar$ interference on ^{80}Se. The medium and high resolution modes of the mass spectrometer were checked. However the medium resolution mode was favored because it provided a higher sensitivity than in high-resolution mode and would not cause interference for As analysis. Optimum sensitivity and signal stability after coupling to the HPLC were achieved by adjusting the nebulizer gas slightly (typical setting of 1.0 L min^{-1}). The signal intensity (based on 10 µg L^{-1} standard) for the monitored isotopes are listed in Table 4.2.

Table 4.1 Review of various chromatography methods of simultaneous separation of inorganic species of As, Sb and Se.

	Matrix	Column	Eluent	Species	Method Information	Reference
As **Se**	Sediment extracts	Anion exchange Hamilton PRX-X100 (250 mm × 4.1 mm, 10 μm)	Gradient elution: A: 10 mM $NH_4H_2PO_4$ pH = 6 (with ammonia) B: 200 mM $NH_4H_2PO_4$ pH = 6 (with ammonia)	As(III), As(V) MA(V), DMA(V), Se(IV), Se(VI)	Flow = 1.0 mL min^{-1} Injection volume = 100 μL Time: < 10 min LOD*: 2 - 40 ng g^{-1} Spike recovery: 80-120%, (except As)	Orero Iserte et al., 2004
As **Se** **Sb**	Standards	Dionex IonPac AS14, (250 mm × 4 mm) with IonPak AG 14, (50 mm × 4 mm)	Gradient elution: A: 2 mM ammonium hydrogen carbonate + 2.2 mM tartaric acid pH = 8.2 (with ammonia) B: 2 mM ammonium hydrogen carbonate + 45 mM tartaric acid pH = 8.2 (with ammonia)	As(III),As(V), MA(V), DMA(V), Se(IV), Se(VI), Sb(III), Sb(V)	Flow = 1.0 mL min^{-1} Injection volume = 50 μL Time: ≈ 15 min LOD*: 4.5 μg L^{-1} (Sb(III) and Se species) 0.5 μg L^{-1} (others) RSD#(retention time): < 2%	Lindemann et al., 1999
As **Sb**	Hot spring water, fish sample	Develosil C30-UG-5 (250 mm × 4.6 mm, 5 μm)	Isocratic elution: 10 mM sodium butanesulfonate + 4 mM malonic acid + 4 mM tetramethylammonium hydroxide + 0.1% (v/v) methanol 20mM ammonium tartrate pH = 2.0	As(III) As(V) MA(V) DMA(V) AB AC TMAO TeMA Sb(III) Sb(V)	Flow = 0.75 mL min^{-1} Injection volume = 10 μL Time: ≈ 12 min LOD*: 0.2 ng mL^{-1} (As) 0.5 ng mL^{-1} (Sb) RSD#: < 2% and 3% (for As and Sb)	Morita et al., 2007
As **Sb** **Se**	Water samples	Anion exchange Hamilton PRX-X100 (250 mm × 4.1 mm, 10 μm)	Isocratic elution: 12.5 mM $(NH_4)_2HPO_4$ + 3% (v/v) methanol pH = 8.5 (with NH_4OH)	As(III) As(V) MMA DMA Sb(V) Se(IV) Se(VI)	Flow = 1.5 mL min^{-1} Injection volume = 100 μL Time: ≈ 20 min	Guerin et al., 1997

AC: arsenocholine; TMAO: trimethylarsine oxide; TeMA: tetramethylarsonium ion; MA: methylarsonate; DMA: dimethylarsinate; AB: arsenobetaine;
* Limit of detection
# Relative standard deviation

4.2.1.2 Separation

The high-pressure liquid chromatographic separations were carried out using a Thermo Scientific Accela 1250 Pump and an Hamilton PRP-X100 (Hamilton, Reno, USA) anion-exchange column (250 mm × 4.1 mm, 10 µm) at a constant flow rate of 1.5 mL min^{-1}. The instrumentation further consisted of a six-port injection valve and a 50 µL sample loop. The HPLC column was connected via a capillary tube (EzyFit Nebulizer sample Tubing) to a Conikal Nebulizer (G.E.). The chromatography conditions are listed in Table 4.3. The pHs of all solutions were determined using a pH-meter (pH 340, WTW).

Table 4.2 The ICP-MS conditions used in the measurement.

Nebulizer	Conikal nebulizer (G.E.)
Nebulizer gas	Around 1.0 L min^{-1}
Spray chamber	Scott type double pass spray chamber (G.E.)
Resolution mode	High resolution (HR), Medium resolution (MR)
Monitored isotopes	^{75}As, ^{78}Se(or ^{82}Se), ^{121}Sb
Signal intensity (cps/10 µg L^{-1})	MR: ^{75}As: 6.0 × 10^4; ^{78}Se: 1.6 × 10^4; ^{121}Sb: 1.2 × 10^5 HR: ^{75}As: 1.0× 10^4; ^{78}Se: 3.3 × 10^3; ^{121}Sb: 3.0 × 10^4

Table 4.3 The chromatography conditions used during analysis.

Column	PRP-X100 (250 mm × 4.1 mm, 10 µm) (Hamilton, Reno, USA)
Mobile phase	0 - 4.5 min: 5 mM EDTA (97%) + methanol (3%) 4.5 - 5.5 min: linear ramp to 30 mM EDTA (97%) + methanol (3%) 5.5 - 11 min: 30 mM EDTA (97%) + methanol (3%)
pH	4.7 (adjusted with Formic acid)
Flow rate	1.5 mL min^{-1}
Injection volume	50 µL
Species	As (III, V), Sb (III, V), and Se (IV, VI)

4.2.2 Reagents and solutions

All solutions were prepared with double deionized water obtained from a Millipore water purification system (MilliQ Advantage A10, 18 MΩ cm).

Stock solutions (1000 mg L^{-1} for each species) were prepared as follows: As(III) from As(III) oxide (As_2O_3, p.a., ACS, Reag. ≥ 99.0% Sigma-Aldrich) dissolved in 4 g L^{-1} NaOH (ACS, Reag. Merck) and preserved in 2% HCl. As(V) from sodium arsenate dibasic heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$, ACS reagent, Sigma-Aldrich) dissolved in water. Sb(III) from potassium antimonyl tartrate trihydrate ($C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$, ACS, Reag. ≥ 99%, Sigma-Aldrich) dissolved in water. Sb(V) from potassium hexahydroxoantimonate (H_6KO_6Sb, for the precipitation of sodium, ≥ 99.0%, Fluka) dissolved in water. Se(IV) from sodium selenite (Na_2O_3Se, 99%, Sigma) dissolved in water. Se(VI) from sodium selenate (Na_2O_4Se, p.a., ≥ 98.0%, Sigma-Aldrich) dissolved in water. All the stock solutions were kept at 4 °C in the dark and analytical standards were prepared daily by appropriate dilution.

The mobile phase was prepared using EDTA (p.a. AppliChem), the pH of which was adjusted with ammonium (Suprapur, Merck) and formic acid (ACS, Reag. 98-100%, Merck). Other acids such as acetic acid (for synthesis, 99-100%, Merck), phosphoric acid (Suprapur, 85%, Merck) and sulfuric acid (GR for analysis, 95-97%, Merck) were also checked. The mobile phase was filtered through a 0.45 μM membrane (Whatman) before use. To enhance plasma performance methanol (for HPLC, ≥ 99.9%, Sigma-Aldrich) was added to the mobile phase.

SRM 1643e (NIST, National Institute of Standards and Technology) and CRM-SW (High-purity Standards) were used as certified reference materials.

4.3 Procedure

As a first step As (As(III) and As(V)) and Sb (Sb(III) and Sb(V)) were studied separately under different chromatographic conditions (*e.g.* pH and concentration of mobile phase), with the purpose of exploring information regarding the influence of pH and concentration of the different mobile phases on the retention times. With the knowledge that EDTA works well for the separation of Se (Wolf et al., 2008), EDTA was the eluent of choice. By changing the concentration, flow rate and pH of the EDTA-based mobile

phase retention times for As(III), As(V), Sb(III) and Sb(V) were obtained first individually and then later in combination for As(III, V) and Sb(III, V), and eventually As(III, V), Sb(III, V) and Se(IV, VI). The chromatographic conditions were optimized by adjusting the pH slightly with different acids. Phosphoric acid, sulfuric acid, acetic acid and formic acid were tested for the adjustment of pH. To further optimize separation and detection various solvent gradients and different methanol concentrations (*e.g.* 1%, 2% and 3% methanol) were investigated. Those separation and detection conditions deemed most efficient were then validated and tested for stability of retention time, linearity, detection limit and recovery. Finally, the method was applied to the analyses of two hot spring samples from Indonesia labeled as J52 and J54, and two CRMs: SRM 1643e and CRM-SW.

4.4 Results and discussion

4.4.1 Development of the speciation analysis method

4.4.1.1 Speciation of As and Sb

Theoretically, the anions and non-charged molecules of a given elemental species, such as $H_3AsO_3^\circ$ and $H_2AsO_4^-$, can be separated in an ion-exchange column. This is caused by differences in their charges, charge densities and distribution of charge on their surfaces, which results in different degrees of binding with the ion exchanger. These binding abilities can be controlled by varying the chromatographic conditions, particularly ionic strength (*e.g.* concentration of mobile phase) and pH. Sepciation of Sb, however, adds additional complexity, because $Sb(OH)_3^\circ$ normally precipitates in the colum. Thus a particular mobile phase is needed in order to stabilize $Sb(OH)_3^\circ$ in solution. Since Sb has a more complexing chromatographic behavior than As and Se, we chose EDTA as a starting point for the optimization of As and Sb species separation, with the idea that EDTA could chelate Sb(III) and form a Sb(III)-EDTA anion. The first step was to investigate retention time as a function of mobile phase concentration. Concentrations of 2 mM, 5 mM, 8 mM, 10 mM and 20 mM were tested on an anion-exchange PRP-X100 (250 mm × 4.1 mm, 10 µm) column. The pH of mobile phase was adjusted to 4.5. The result showed that, the retention times of As(III) and Sb(V) more or less remained constant in the concentration range from 2 mM to 20 mM, while on the other hand, the retention times of As(V) and Sb(III) decreased, particularly for Sb(III), whose retention time decreased sharply from 2 mM to 10 mM. Considering the interaction of Sb(III) and

EDTA, it makes sense that the retention time of Sb(III) was more dependent on the concentration of EDTA. Taking both, resolution and retention time, into consideration, 5 mM seemed to be the optimum concentration of EDTA in the mobile phase, which allowed a relatively short analysis time and good separation of the chromatographic peaks of As(V) and Sb(III). For the flow rate of mobile phase, 1.5 mL min^{-1} was favored, as previous work (Ruiz-Chancho et al., 2013) had shown that 1.5 mL min^{-1} worked well for the As(III) and As(V) speciation analysis on an Hamilton PRP-X 100 anion-exchange column. A solution containing 10 µg L^{-1} of each As(III), As(V), Sb(III) and Sb(V) was analyzed with 5 mM EDTA as mobile phase (pH = 4.5). The chromatogram showed that each analyte had a good separation, while the separation as a whole was finished in 16 min.

Our chromatogram revealed an additional peak at around 15 min, which following Hansen et al. (2011) was interpreted to be that of Sb(V)-polymer. Since EDTA can not chelate Sb(V), and thus Sb(V)-polymer and $Sb(OH)_6^-$ should elute at different times. Hansen et al. (2011) assumed that the inability to convert Sb(V) complexes into one common complex may partly explain the emergence of unidentified peaks in Sb speciation analysis (Miekeley et al., 2002; Hansen and Pergantis, 2007; Foster et al., 2005). They suggested acidic hydrolysis of samples with 1M HCl in the presence of chelating ligands such as EDTA and citrate. The same phenomenon was also verified when EDTA and phthalic acid combined were used as mobile phase (Amereih et al., 2005). In this study whenever the unidentified Sb(V) peak appeared in a chromatogram, it was integrated together with the Sb(V) peak for the calculations of Sb(V) linearity and recovery.

4.4.1.2 Speciation of As, Sb and Se

The chromatographic conditions obtained in the first step were subsequently applied for the separation of Se(IV) and Se(VI). However, the result revealed that Se speciation has a strong dependency on the acid used for pH control. Thus different acids, such as phosphoric acid, sulfuric acid, acetic acid and formic acid were tested for the adjustment of pH of the mobile phase. Eventually phosphoric acid, sulfuric acid and acetic acid were discarded, because they (specially phosphoric acid) led to a surprisingly high baseline and poor peak shape for Se(IV) and Se(VI). While we did not investigate this further one could assume that the purity of those acids may have played a role. Once formic acid

was chosen as the appropriate acid separation and retention were optimized by adjusting the pH. Because pH can affect the species retention time by changing the existing forms of eluent and solute ions, a solution containing 10 µg L^{-1} of As(III), As(V), Sb(III), Sb(V) and 100 µg L^{-1} of Se(IV) and Se(VI) was analyzed using 5 mM EDTA as a mobile phase in the pH range from 4 to 5. The result revealed that the retention times of Se(VI) and the unidentified peaks for a Sb(V)-polymer were shortened by increasing the pH from 4 to 5, while other species remained more or less the same position, indicating less dependence on pH. The behavior of Se(VI) could be explained solely based on the physicochemical properties of the eluent. EDTA is known for its various protonated forms depending on pH; from H_6EDTA^{2+} at very acidic condition to $EDTA^{4-}$ at very basic conditions. It could function as a powerful competing anion and thus has a strong influence on polyvalent anions such as SeO_4^{2-}. The Sb(V)-polymer was affected greatly by pH, probably due to hydrolysis and change of charged situation of Sb(V)-polymer in response to the change in pH. Increasing pH shortened of the total analysis time, but at the same time causing deterioration of the Sb(III) peak shape. Noteworthy, the "unwanted" peak of Sb(V)-polymer became larger with increasing pH, an observation in accordance with Hansen et al. (2011) who postulated that the Sb(V)-polymer peak would become smaller at a lower pH because of acidic hydrolysis. Compromisingly, a pH value of 4.5 was favored as the optimum acidic condition for the mobile phase.

4.4.2 Optimization of chromatographic conditions

4.4.2.1 Addition of methanol

Methanol is one of the most commonly used organic compounds for modification of chromatographic conditions, because it can be used to improve signal intensity during ICP-MS detection and changes retention time during speciation analysis (Guerin et al., 1997; Ulrich, 1998). Thus, the logic next step in method development was to investigate the potential to improve separation and detection by adding methanol to the mobile phase. The result was that signal intensity was enhanced greatly for As (As(III) and As(V)) and Se (Se(IV) and Se(VI)) when methanol was added to the mobile phase. The intensity for Sb species, however, was only slightly improved. A suggested explanation for this phenomenon is that the loading of carbon-containing polyatomic ions into the plasma leads to a strongly increased population of C^+ and/or carbon-containing polyatomic ions and the degree of ionization of a given analyte is improved by transfer of

electrons to the carbon ions (or other carbon-containing ions) from that analyte (Larsen and Stürup, 1994). This would indicate that As and Se species were not fully ionized in the plasma. A combination of 3% methanol and 5 mM EDTA provided the optimum chromatographic conditions although it slightly increased analysis time. To offset this increase the mobile phase pH was changed from 4.5 to 4.7. This shortened the overall analysis time, without remarkable effect on the quality of the peak shapes. The chromatogram obtained under what was considered optimum chromatographic conditions (5 mM EDTA in combination with 3% methanol at pH of 4.7 adjusted with formic acid) showed that all species had a good signal sensitivity and detection was finished in just under 22 min. These species eluted in the order of As(III), Sb(V), As(V), Se(IV), Sb(III) and Se(VI).

4.4.2.2 Application of a solvent gradient

The first four peaks (As(III), Sb(V), As(V) and (Se(IV)) were sufficiently separated and could be eluted in the first 4 min after injection. However, the remaining peaks for Sb(III) and Se(VI) came much later; at 8.3 min for Sb(III) and 19.1 min for Se(VI), indicating that they were strongly retained in the column, which can also be seen from their broad peak shape and long retention time, thus leading to a pretty long overall analysis time of more than 22 minutes. In order to shorten the separation time and to improve the detection limit of Sb(III) and Se(VI), a further modification was made by applying 30 mM EDTA combined with 3% methanol as a solvent gradient which is a common strategy in chromatography (Müller et al., 2009). The gradient program was: 0 – 4.5 min, 97% 5 mM EDTA and 3% methanol; 4.5 – 5.5 min, linear ramp to 97% 30 mM EDTA and 3% methanol; 5.5 – 11min, 97% 30 mM EDTA and 3% methanol. With this gradient setting the first four peaks were kept in their original position and shape, while the two remaining peaks had improved elution time and peak shape. Thus, the sensitivity of Sb(III) and Se(VI) was improved which ensured a lower detection limit and shortened the overall analysis time to 11 min. Despite the variation in composition of the mobile phase, no shift of the base line was observed, which could be a byproduct of applying a gradient during HPLC separation. A chromatogram of a standard solution containing 10 µg L^{-1} As(III,V) and Sb(III,V), and 100 µg L^{-1} Se(IV,VI) is presented in Fig. 4.1.

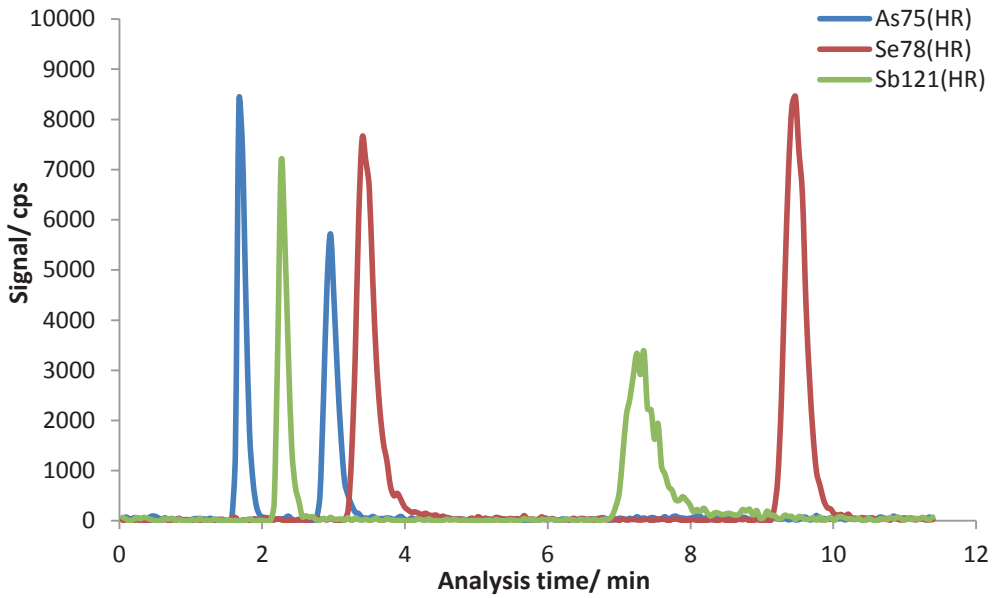

Fig. 4.1 Chromatogram of a standard containing 10 µg L^{-1} As(III,V) and Sb(III,V), and 100 µg L^{-1} Se(IV,VI); The peaks from left to right are As(III), Sb(V), As(V), Se(IV), Sb(III) and Se(VI) respectively.

4.4.3 Validation

Following development a complete evaluation was conducted to evaluate stability of retention time, linearity, detection limit and spike recovery of the method.

4.4.3.1 Stability of retention time

To determine the retention times for the six species replicate measurements were made. The relative standard deviation (RSD) was calculated in order to check its stability (Table 4.4. Mean ± RSD, n ≥ 20). Table 4.4 shows that the retention times for As(III), As(V), Sb(III), Sb(V), Se(IV) and Se(VI) were 1.70, 2.94, 7.14, 2.28, 3.38 and 9.36 min, respectively. Low RSD of 2% for As(III) and Sb(V) and 4% for As(V) and Se(IV) were obtained from more than 20 replicate measurements. Even after the eluent gradient was introduced, Sb(III) and Se(VI) still had RSDs of less than 10% (8% for Sb(III) and 9% for Se(VI)).

4.4.3.2 Detection limit

The limit of detection (LOD) was calculated according to the recommendation of the IUPAC (International Union of Pure and Applied Chemistry), as the corresponding concentration of 3 times the standard deviation for the signal/noise (S/N) ratio for each species. Blank solution (Milli-Q water) spiked with concentrations close to the detection limit of all six desired species (0.2 μg L^{-1} for As(III,V) and Sb(III,V), and 0.5 μg L^{-1} for Se(IV,VI)) were measured 11 times to calculate the corresponding detection limits. Fig. 4.2 showed the chromatography close to the detection limit. The detection limits for As(III), As(V), Sb(III), Sb(V), Se(VI) and Se(IV) were 0.02 μg L^{-1}, 0.06 μg L^{-1}, 0.2 μg L^{-1}, 0.02 μg L^{-1}, 0.2 μg L^{-1} and 0.4 μg L^{-1} respectively (Table 4.4), which in general were better than the LODs for those methods listed in Table 1. In addition to S/N, peak height and peak area for each species were also calculated to confirm their detection limits and similar results were obtained.

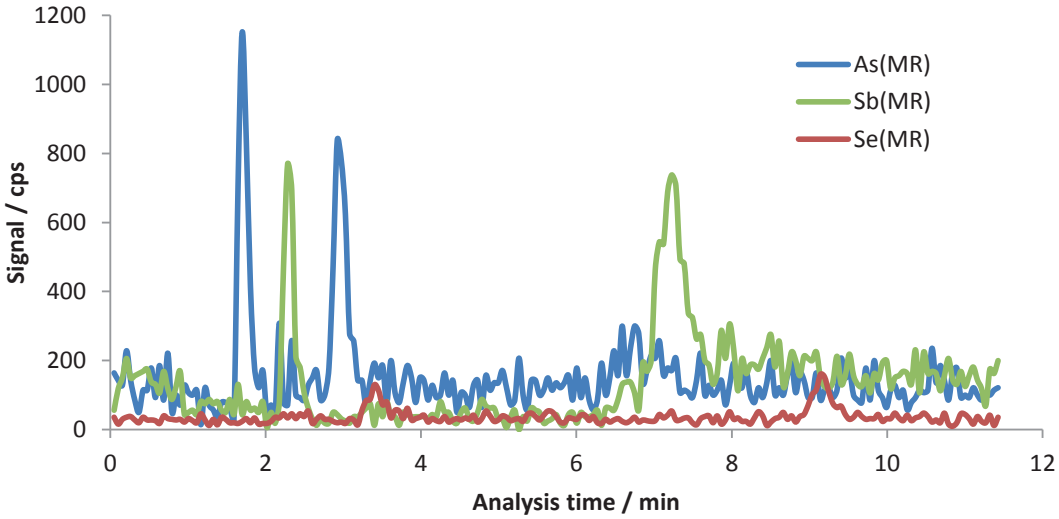

Fig. 4.2 Chromatogram of a standard containing 0.2 μg L^{-1} As(III,V) and Sb(III,V), and 0.5 μg L^{-1} Se(IV,VI); The peaks from left to right are As(III), Sb(V), As(V), Se(IV), Sb(III) and Se(VI) respectively.

4.4.3.3 Linearity

The linearity of each calibration curve was examined for different concentration ranges with at least 5 standard points. The linearity for As(III, V) and Sb(III, V) was investigated

for the concentration range from 0.5 to 75 µg L^{-1}, and the range from 5.0 to 200 µg L^{-1} was used for Se(IV, VI), which are the regulatory concentration ranges for many different water types, including ground water (Plant et al., 2006; Filella et al., 2002a). The results showed that detection in these concentrations ranges was linear, as demonstrated by excellent correlation coefficients. The linear correlation coefficients (R^2), obtained from at least 6 replicate experiments, were 0.9999 for As(III) and Se(IV), and 0.9998 for As(V) (Table 4.4).

Table 4.4 The concentration range, correlation coefficient, detection limit and retention times for six species. The correlation coefficients were obtained from at least 6 replicate experiments. The detection limit was calculated from 11 replicate measurements of blank. The retention times were obtained from at least 20 replicate measurements.

	Concentration range / µg L^{-1}	Correlation coefficient / R^2	Limit of detection (LOD) / µg L^{-1}	Retention time / min
As(III)	0.5 - 75	0.9999	0.02	1.70 ± 0.02
As(V)	0.5 - 75	0.9998	0.06	2.94 ± 0.04
Sb(III)	0.5 - 75	0.9998	0.2	7.14 ± 0.08
Sb(V)	0.5 - 75	0.9998	0.02	2.28 ± 0.02
Se(IV)	5.0 - 200	0.9999	0.2	3.38 ± 0.04
Se(VI)	5.0 - 200	0.9998	0.4	9.36 ± 0.09

4.4.3.4 Recovery

To our knowledge, only a few methods focused on the simultaneous analysis of As, Se and Sb species in fluid samples have been reported. In the absence of a certified reference material (CRM), which contains As(III, V), Sb(III, V) and Se(IV, VI) the strategy was to carry out spike recovery experiments for each species, which enabled us to perform a quantitative evaluation of the method. The chromatographic recovery for every individual species was estimated by calculating the quantity of the species eluted from the column as a percentage of the amount injected into the column.

Firstly, a synthetic sample prepared with deionized water with low concentrations approximately from 1.0 to 2.5 µg L^{-1} for all species were analyzed. A duplicate sample was spiked with 1.0 µg L^{-1} As(III, V), Sb(III, V) and 2.0 µg L^{-1} Se(IV, VI). Both the

synthetic sample and the spiked one were measured 3 times and the spike recovery was calculated (Table 4.5). Subsequently, spike recoveries were tested using two “real” samples labeled as J52 and J54. The two samples were from two hot springs in Cisolok and Patuha on Java Island, Indonesia. The initial temperature and pH for sample J52 in the field were 102.0 ℃ and 8.1 and the initial temperature and pH for sample J54 were 32.9 ℃ and 1.0. Both J52 and J54 were chloride rich samples; 305.6 mg L^{-1} for J52 and 35.2 mg L^{-1} for J54. The samples were acidified with HCl and preserved in the dark at room temperature after sampling. These two samples were diluted 5 fold with deionized water prior to spiking to bring the concentrations into the calibration and linearity range of the method. For sample J52, 20 μg L^{-1} As(III, V), 1.0 μg L^{-1} Sb(III, V) and 5.0 μg L^{-1} Se(IV, VI) were added as spikes. For sample J54, 5 μg L^{-1} As (III), Se(IV, VI) and 0.5 μg L^{-1} Sb(III, V), As(V) were added. Each sample (with and without spikes) was measured at least 3 times and the results are listed in Table 4.5. It can be seen that: (i) Except the recovery of As(III) for synthetic sample (89%) and Sb(III) for sample J52 (123%), all the other species had a spike recovery of 90 - 110%, indicating that almost no mass loss happen in chromatography. (ii) In the two analyzed hot spring samples, the concentration of As is much higher than Sb and Se. In sample J52 only As(V) and Sb(V) were detectable probably because of oxidation caused by inappropriate preservation. (iii) Although, in the two hot spring samples no Se species were determined. However, the good recovery (98.8% and 107.6% for Se(IV), and 97.8% and 109.4% for Se(VI)) indicated that the proposed method could be successfully applied to Se(IV, VI) speciation analysis in real samples.

Table 4.5 Spike recovery of a synthetic sample and two hot spring samples J52 and J54. The data was obtained from at least 3 replicate measurements for each sample.

	Synthetic sample			Sample J52			Sample J54		
	Spike ($\mu g\ L^{-1}$)	Determined ($\mu g\ L^{-1}$)	Recovery (%)	Spike ($\mu g\ L^{-1}$)	Determined ($\mu g\ L^{-1}$)	Recovery (%)	Spike ($\mu g\ L^{-1}$)	Determined ($\mu g\ L^{-1}$)	Recovery (%)
As(III)	0.0	1.03 ± 0.02	—	0.0	0	—	0.0	3.89 ± 0.02	—
	1.0	1.92 ± 0.01	89.0	20.0	18.83 ± 0.83	94.2	5	8.59 ± 0.31	94
As(V)	0.0	1.21 ± 0.01	—	0	19.45 ± 0.56	—	0.0	0	—
	1.0	2.11 ± 0.01	90	20.0	38.83 ± 0.70	96.9	0.5	0.51 ± 0.01	102
Sb(III)	0.0	1.58 ± 0.02	—	0	0	—	0.0	0.40 ± 0.01	—
	1.0	2.53 ± 0.03	95	1.0	1.23 ± 0.09	123	0.5	0.90 ± 0.00	100
Sb(V)	0.0	1.56 ± 0.01	—	0	0.83 ± 0.06	—	0.0	0.38 ± 0.02	—
	1.0	2.62 ± 0.07	106	1.0	1.77 ± 0.11	94	0.5	0.87 ± 0.01	98
Se(IV)	0.0	2.21 ± 0.06	—	0	0	—	0.0	0	—
	2.0	4.10 ± 0.06	94.5	5.0	4.94 ± 0.59	98.8	5	5.38 ± 0.04	107.6
Se(VI)	0.0	2.08 ± 0.01	—	0	0	—	0.0	0	—
	2.0	3.95 ± 0.06	93.5	5.0	4.89 ± 0.20	97.8	5	5.47 ± 0.08	109.4

4.4.4 Application and matrix interference

The proposed method was finally applied to two CRMs: SRM 1643e (NIST, National Institute of Standards and Technology) and CRM-SW (High-purity Standards). It is worth noting that in the CRM-SW no Sb species were present. The two CRMs were measured without dilution, and 3 replicate measurements were carried out. In SRM 1643e As(V)

was predominant and only trace amounts of As(III) were detected. In the case of Sb and Se, only Sb(V) and Se(IV) were detected. In CRM-SW, only As(V) and Se(IV) were detected. The results were in good agreement with the certified values (Table 4.6).

Matrix interferences were evaluated, including the potential $^{40}Ar^{35}Cl^{+}$ interference on As analysis, and the matrix effect on species retention time and results. In order to check if Cl^{-} can cause interference for As species, a standard solution containing 10 μg L^{-1} of the six desired species and 500 μg L^{-1} Cl^{-} was injected into the column. The result showed that Cl^{-} was eluted at 6.11 min, indicating that it would not cause any interference for As analysis because As(III) and As(V) were eluted at 1.70 and 2.94 min respectively. The retention times of the six species were evaluated again when analyzing the two hot spring samples and the two CRMs. The results showed no shift for those species detected in the hot spring samples. In SRM 1643e and CRM-SW, the detected species generally showed a slightly earlier elution than the standards. However, no obvious matrix influence could be inferred.

Table 4.6 The results of certified reference materials SRM 1643e and CRM-SW. Data obtained from 3 replicate measurements.

Sample	Species	Determined (μg L^{-1})	Certified (μg L^{-1})
SRM 1643e	As(III)	0.62 ± 0.07	60.45 ± 0.72
	As(V)	58.03 ± 0.87	
	Sb(III)	ND	58.30 ± 0.61
	Sb(V)	58.40 ± 2.63	
	Se(IV)	11.85 ± 0.51	11.97 ± 0.14
	Se(VI)	ND	
CRM-SW	As(III)	ND	20.00 ± 0.00
	As(V)	20.80 ± 1.40	
	Se(IV)	4.42 ± 0.39	4.00 ± 0.00
	Se(VI)	ND	

ND: not determined.

4.5. Conclusion

In this paper, a new simultaneous speciation analysis method focused on inorganic redox species of As(III, V), Sb(III, V), and Se(IV, VI) based on HPLC-SF-ICP-MS was developed using an anion-exchange column. EDTA combined with 3% methanol was used as mobile phase. The total analysis time was less than 11 minutes by introducing in a solvent gradient. The inlet system of detection consisted of a scott type double pass spray chamber and a conikal nebulizer. All species were measured free of interference. Linear correlation coefficients of ≥ 0.9998 for all calibration curves were obtained. The method showed a low detection limit, generally lower than related report, for each desired species. Most species in the analyzed samples had a spike recovery of 90 - 110%. No inter-conversion between species or mass loss during chromatography was observed. The study confirmed the complexing property of Sb that Sb(III) rather than Sb(V) had a strong affinity in the column, and Sb(V) existed in the form of $Sb(OH)_6^-$ and Sb(V)-polymer. The method is characterized with simple eluent composition, short overall analysis time, low detection limit, good linearity and reliable repeatability of retention time, and thus could be safely applied to a variety of fluid samples.

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5. Preservation and stability of As, Sb and Se redox couples in water samples

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Abstract

The simultaneous preservation of the following redox couples was studied: As(III, V), Sb(III, V) and Se(IV, VI). Over a time period of 11 weeks the stability of these three redox couples was assessed in groundwater, lake water and river water using different preservation strategies. High concentrations of Fe (25.0 mg L^{-1}) and Mn (25.0 mg L^{-1}) were added to each of the different matrices to simulate a Fe and Mn rich environment. In addition to their natural concentration, each sample was spiked with 5.0 µg L^{-1} As(III and V) and Sb(III and V) and 15.0 µg L^{-1} Se(IV and VI). As potential preservation strategies EDTA alone and EDTA combined with either HCl, HNO_3, formic acid or acetic acid were investigated and compared to unpreserved samples. In addition preserved samples were stored at 4 °C in the dark, while unpreserved samples were stored at room temperature in the presence of light. The results showed that addition of EDTA combined with acidification to a pH of 3 successfully preserved all three redox couples for at least 11 weeks stored at 4 °C in the dark. EDTA alone (pH = 6) failed to preserve the As and Sb species, although it successfully preserved the Se species. Primarliy based on observations made for the unpreserved samples it was concluded that Sb(III) could be oxidized easier than As(III) and Se(IV) at neutral pH, and that the Se species in general were most stable. The formation of Fe-(oxy) hydroxide and possibly Mn-(oxy) hydroxide in the unpreserved samples also allowed an estimation of the relative adsorption behavior. As(III), Sb(III), Se(IV)) and As(V) showed a strong adsorption affinity for Fe-(oxy)hydroxide and/or Mn-(oxy)hydroxide probably due to the fact that they all form inner sphere complexes. While Sb(V) and Se(VI) were not adsorbed in most cases because they form outer sphere complexes and thus bonded via weak electrostatic adsorption. Sb(III) could chelate with EDTA and formed several complexes according to pH. The most stable species of $Sb(III)Y^-$ (Y = EDTA) existed at a pH range of 1.8 to 3.0. Apparently Sb(V), on the other hand, did not chelate with EDTA and thus should exist mainly in the form of $Sb(OH)_6^-$ and minor $Sb(OH)_5$ at this pH range.

5.1 Introduction

Redox sensitive, multi species elements, such as arsenic (As(III, V)), antimony (Sb(III, V)) and selenium (Se(IV, VI)) were intensively studied, because they play an important role in both environmental and health issues. To date great effort was made to investigate the stability and preservation strategies for individual element species(Kumar and Riyazuddin, 2010), however the next step will be to study the behavior of various element species in different environments simultaneously (Lindemann et al., 2000). For example, redox behavior of these elements in different matrices (Breuer and Pichler, 2013; Lazareva et al., 2015; Leuz, 2006; Wallis et al., 2011), toxicity and bioavailability as a function of redox state (Jamier et al., 2010; Price et al., 2013; Mailloux et al., 2009; Price et al., 2007) and competitive adsorption of, *e.g.* arsenic and antimony, onto hydrous ferric oxide (HFO) surfaces (Qi and Pichler, 2014). Up to now the diverse properties of these species are still not fully understood. The main reason might be the lack of methods allowing simultaneous speciation analysis of these redox couples and the difficulty to preserve the distribution of these species from sampling to measurement. The stability of these species depends on redox condition, pH, microbial activity, photochemical oxidation, organic matters, presence of oxidizing ions like Fe(III), (oxy)hydroxide co-precipitation, adsorption on container walls and particles like ferrihydride. As a matter of fact published preservation methods for the three elements of interest are contradictory.

Gómez-Ariza et al. (1998) carried out a systematic investigation about the variables that can affect the stability of inorganic selenium species, including species concentration, pH, container material, temperature and matrix (seawater and fresh water *e.g.* river and tap water). The result showed that when the samples were acidified to pH = 2 with HCl and stored at -20 °C in Teflon containers, Se(VI) remained stable for a whole year in all studied matrices. As for Se(IV), clear losses were observed in fresh water (river and tap water) after 6 months. However, in seawater samples Se(IV) was stable for a year, indicating a higher stability at high ionic strengths. They also found that Se(VI) was more stable than Se(IV) and higher concentrations were more stable than lower. To the contrary, Cobo et al. (1994) suggested that acidification was not necessary for preserving inorganic Se species at -20 °C. Se(IV) was more stable at pH 6 than at pH 4, with two months being the maximum storage time without Se(IV) loss. Besides, Héninger et al. (1997) pointed out that HCl acidification may catalyze the oxidation of

Se(IV) to Se(VI). Wiedmeyer and May (1993) studied the influence of ionic strength, container material, temperature on the stability of Se species. Compared to Se(VI), significant changes of Se(IV) were observed over 120 days, and loss of Se(VI) was observed in a low ionic strength matrix. The least change during storage was observed at 4 °C in a glass container.

Preservation of inorganic Sb species seems more complicated than As and Se, especially for time spans in excess of one month, because Sb forms strong complexes and its species are easier affected by adsorption and oxidation. Acidification with HCl proved to be ineffective. Andreae (1983) observed a rapid oxidation of Sb(III) in estuarine water samples preserved with HCl acidification, but total Sb remained stable for several months. Oxidation of Sb(III) was also observed in seawater acidified with HCl by Cabon and Louis Madec (2004) and Ellwood and Maher (2002). Freezing is somewhat controversial to preserve Sb species. Andreae (1983) observed fast oxidation in the brine formed during partial freezing of estuarine water samples. Cutter and Cutter (1995) found that quickly frozen samples could not be stored longer than one month before the inorganic speciation was compromised. Besides, stabilizing agents such as tartaric acid and EDTA were also investigated and found to prevent oxidation of Sb(III) for six days (Han-wen, 1982; Gregori et al., 2005).

As(III, V) preservation was most widely studied among the three elements of interest. Some reviews summarized and evaluated the published methods (Mccleskey et al., 2004; Kumar and Riyazuddin, 2010). However, there are still discrepancies regarding preservation of As species (Mccleskey et al., 2004). Acidification is one of the most common procedures for preserving As species, however, Bednar et al. (2002) observed an immediate oxidation of As(III) in synthetic samples preserved with HCl in both dark and light conditions. Hall et al. (1999) studied the stability of inorganic As(III) and As(V) in spiked de-ionized water and river water which were acidified with HCl or HNO_3, and the result showed that both HCl and HNO_3 caused oxidation of As(III) to As(V), but HNO_3 led to a higher degree of oxidation. EDTA combined with acetic acid were also investigated as potential preservation for As species (Samanta and Clifford, 2006; Gallagher et al., 2004; Wang and Liu, 2012).

According to the conditions leading to the change of distribution of different species, the preservation of these redox species should be discussed considering the following aspects.

1. Filtration. It is a common practice to filter with a 0.20 μm or 0.45 μm membrane for sample preservation, as it excludes suspended particles and microorganisms. Because bacteria are capable to catalyze As and Sb oxidation (Asta et al., 2012).
2. Acidification. Most researchers prefer acidification, because low pH (< 3) impedes the hydrolysis of Fe(III). Fe(III) is abundant in the environment and one of the major factors that can affect the distribution of redox couples of As, Sb and Se by forming Fe-(oxy)hydroxide or leading to oxidation of these species. A variety of acids were studied and acidification alone seemed insufficient. HCl and HNO_3 were discussed controversially. H_2SO_4 and H_3PO_4 worked well in some cases (Daus et al., 2006; Bednar et al., 2002; Daus et al., 2002), but were discarded either due to the fact that they were difficult to purify and might cause precipitation by forming metal phosphate (or sulfate) *e.g.* strengite ($FePO_4 \cdot 2H_2O$).
3. Temperature. Temperature influences microbial activity as well as chemical reactions (endothermic/exothermic). Cooling the water samples to different temperatures, *e.g.* 3 °C, 6 °C and -20 °C was widely investigated for preservation of As, Sb and Se species (Lindemann et al., 2000; Daus et al., 2006). However, long term storage at very low temperature is not practical for sampling in the field and transportation to the lab. Therefore, freezing is a controversial technique leading to precipitation and repartitioning (Héninger et al., 1997).
4. Light. To our knowledge no preservative tested could maintain the As(III/V) speciation when exposed to light When a water sample containing Fe(III) is exposed to light, hydroxyl radicals are produced at pH >2, and dichlor radicals are produced when the sample is acidified with HCl to pH < 2. These radicals react with As(III) to produce intermediate As(IV) species reacting with Fe(III) to produce Fe(II) and As(V) (Mccleskey et al., 2004). An instant switch from As(III) to As(V) was observed by Bednar et al. (2002) with light exposure when hydrochloric acid was used for preservation. In another study Samanta and Clifford (2005) showed that in the presence of strong UV light and Fe(II), neither EDTA-HAc, H_2SO_4 nor H_3PO_4 could prevent the rapid oxidation of As(III). It has been reported that nitrate could also undergo photochemical reaction and

produce hydroxyl radicals (Fanning, 2000). Therefore, samples should be preserved in opaque bottles to eliminate/minimize the influence of UV light.

5. (Oxy)hydroxide adsorption and precipitation. Fe-(oxy) hydroxide and/or Mn-(oxy) hydroxide can bind various trace elements, including As, Sb and Se due to their large surfaces, high adsorptive capacities and large abundance in the environment. Thus, the formation of Fe-(oxy) hydroxide and/or Mn-(oxy) hydroxide should be avoided in any case during storage.

To our knowledge, a preservation strategy for inorganic species of As(III, V), Sb(III, V) and Se(IV, VI) has not yet been reported. With the development of a method for the simultaneous speciation of As(III, V), Sb(III, V) and Se(IV, VI) using SF-ICP-MS coupled to HPLC (Wu and Pichler, 2014) a preservation strategy for As(III, V), Sb(III, V) and Se(IV, VI) is necessary.

5.2 Material and method

5.2.1 Instrumentation

5.2.1.1 Detection

A Thermo Scientific ELEMENT 2 sector field ICP-MS (SF-ICP-MS) was used for the detection of As(III, V), Sb(III, V), and Se(IV, VI). The instrumental conditions and tuning information are given in Table 5.1. For As and Sb, isotopes of ^{75}As and ^{121}Sb were monitored. For Se, the less abundant isotope of ^{78}Se was used due to the $^{40}Ar^{40}Ar$ interference on ^{80}Se. The high resolution mode of ELEMENT 2 was used for analysis (Table 5.1).

The major anions and cations in Table 5.2 were measured using IC (Basic IC plus 883, Metrohm) and ICP-OES (Optima 7300DV, PerkinElmer).

5.2.1.2 Separation

The chromatographic separations were carried out using a Thermo Scientific Accela 1250 Pump and an Hamilton PRP-X100 (Hamilton, Reno, USA) anion-exchange column (250 mm × 4.1 mm, 10 µm) at a constant flow rate of 1.5 mL min^{-1}. The chromatography conditions are listed in Table 5.1. The outlet of the HPLC column was connected via

PEEK capillary tubing to the nebulizer of ELEMENT 2. The pH of all solutions was determined using a pH-meter (pH 340, WTW).

Table 5.1 Instrumental conditions for the simultaneous detection and separation of multiple redox couples.

Nebulizer	Conikal nebulizer
Spray chamber	Scott type spray chamber
Detection mode	High resolution (HR)
Monitored isotopes	^{75}As, ^{78}Se, ^{121}Sb
Column	PRP-X100 (250 mm × 4.1 mm, 10 µm) (Hamilton, Reno, USA)
Mobile phase	0 - 4.5 min: 5 mM EDTA (97%) + methanol (3%) 4.5 - 5.5 min: linear ramp to 30 mM EDTA (97%) + methanol (3%) 5.5 - 11 min: 30 mM EDTA (97%) + methanol (3%)
pH	4.7 (adjusted with formic acid)
Flow rate	1.5 mL min^{-1}
Injection volume	50 µL
Species	As (III, V), Sb (III, V), and Se (IV, VI)

5.2.2 Reagents and solutions

All solutions were prepared with double de-ionized water obtained from a Millipore water purification system (MilliQ Advantage A10, 18 MΩ cm).

Stock solutions (1000 mg L^{-1} for each species) were prepared as follows: As(III) from As(III) oxide (As_2O_3, Sigma-Aldrich) dissolved in 4 g L^{-1} NaOH (Merck). As(V) from sodium arsenate dibasic heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$, Sigma-Aldrich) dissolved in water. Sb(III) from potassium antimonyl tartrate trihydrate ($C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$, Sigma-Aldrich) dissolved in water. Sb(V) from potassium hexahydroxoantimonate (H_6KO_6Sb, Fluka) dissolved in water. Se(IV) from sodium selenite (Na_2O_3Se, Sigma) dissolved in water. Se(VI) from sodium selenate (Na_2O_4Se, Sigma-Aldrich) dissolved in water. Fe(II) (10 000 mg L^{-1}) from iron(II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$, Merck) dissolved in water. Mn(II) (10 000 mg L^{-1}) from manganese(II) chloride dihydrate ($MnCl_2 \cdot 2H_2O$, Merck) dissolved in water. 0.2 M EDTA from EDTA (p.a. AppliChem) dissolved in water. All

stock solutions were kept at 4 °C in the dark and the standards and mix standards of lower concentration were prepared daily by appropriate dilution with water.

The mobile phase was prepared using EDTA (p.a. AppliChem), the pH of which was adjusted with ammonium (Suprapur, Merck) and formic acid (98-100%, Merck). The mobile phase was filtered through a 0.45 µM membrane (Whatman) before use. Methanol (for HPLC, ≥ 99.9%, Sigma-Aldrich) was used in combination with EDTA solution as mobile phase.

5.2.3 Sampling and storage condition

Three aqueous matrices: groundwater (G), river water (R) and lake water (L) containing all six desired species were studied. Immediately after collection, temperature (T), dissolved oxygen (DO), Eh and pH were measured (Table 5.2). The samples were then filtered with a 0.45 µm membrane, and subsequently divided into 6 subsamples of 50 mL. Then 125 µL of a 10000 mg L^{-1} Fe^{2+} and Mn^{2+} stock solution was added to all subsamples to generate a Fe^{2+} and Mn^{2+} rich matrix (25.0 mg L^{-1} for each). In addition 500 µL of 0.2 M EDTA was added to five of the six subsamples. To ensure that all six desired species were present the subsamples were spiked by adding 5.0 µg L^{-1} As(III and V), Sb(III and V) and 15.0 µg L^{-1} Se(IV and VI). It is important that the Fe^{2+} stock solution was deemed as a mix of Fe^{2+} and Fe^{3+}, which can be seen from the brown color rather than pale green of its solution. All subsamples were stored in 50 mL PE bottles. The pH for all above prepared samples was measured prior to storage; about 6.2 for the samples without preservative and with EDTA only. For those preserved with EDTA and acid, the pH was adjusted to around 3 with HCl(Cl), HNO_3(N), formic acid(Fo) and acetic acid(Ac) respectively. The subsamples without any preservation were labeled as Gw/o (Rw/o, Lw/o) and stored under normal conditions (room temperature, with the presence of light). The subsamples preserved with EDTA only were labeled as GE (RE, LE). The subsamples preserved with EDTA combined with acid (HCl, HNO_3, formic acid and acetic acid) were labeled as GClE (RClE, LClE), GNE (RNE, LNE), GFoE (RFoE, LFoE) and GAcE (RAcE, LAcE). Those preserved with EDTA or EDTA combined with acid were stored at 4 °C in the dark (see Fig. 5.1). The samples were measured within hours after collection. Those samples without any preservation changed color to a yellow/reddish brown, indicating that Fe-(oxy)hydroxide and possibly Mn-(oxy)hydroxide precipitated. To verify this assumption, Fe and Mn were measured at the end of the

experiment in the unpreserved samples. The measurements showed that Fe was completely removed from solution and that between 60 and 80 % of the Mn were removed from solution. Those samples preserved with EDTA remained colorless and transparent, indicating that Fe and Mn were chelated by EDTA. The samples without any preservation were filtered with 0.45 µm membrane each time before measurement, due to the dark brown suspension it formed. All the samples were measured periodically; 1 week, 2 weeks, 3 weeks, 7 weeks and 11 weeks. Every time 500 µL of each sample was taken for measurement.

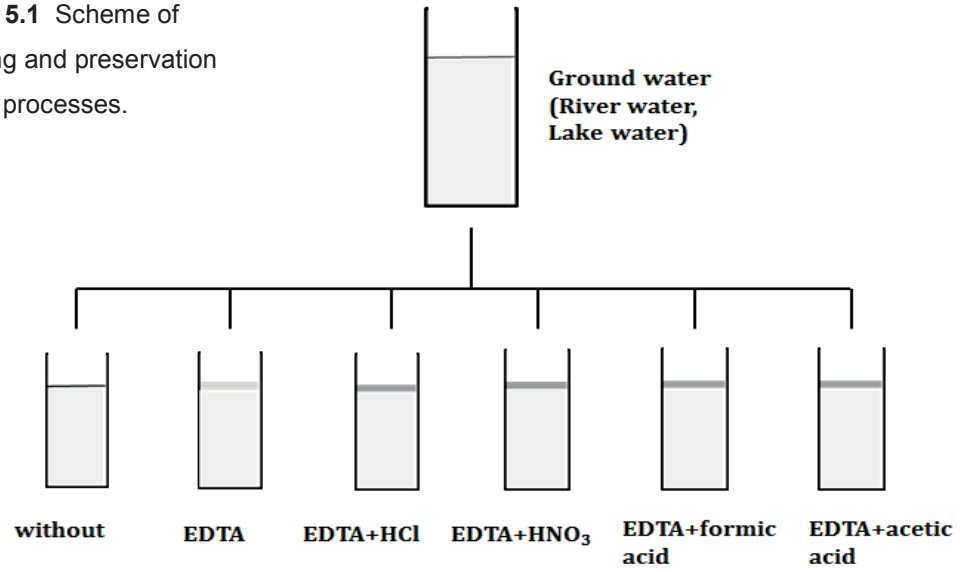

Fig. 5.1 Scheme of sampling and preservation processes.

5.3 Results and discussion

5.3.1 The matrix effect

The matrix plays an important role for the stability of redox sensitive species, such as As, Sb, Se, Mn and Fe. Numerous experiments revealed that the presence of microbial organism, dissolved organic carbon and ionic strength all affect the stability of redox species (Gómez-Ariza et al., 1998). Therefore it is necessary to analyse the matrix as first step (Table 5.2). Groundwater had a lower dissolved oxygen (2.88 mg L^{-1}) and a higher Fe concentration (2.3 mg L^{-1}) than river water and lake water whose Fe concentrations were almost not detectable (Table 5.2). As has been indicated Fe may undergo photochemical reactions and further lead to oxidation of those species. Dissolved oxygen, although it cannot cause remarkable changes to these species alone, may catalyze oxidation of those redox species in combination with Fe. Lake water was more abundant in Ca, Mg and sulfate than groundwater and river water. These ions are known to form minerals once physicochemical conditions change and thus they may cause co-precipitation of As, Sb and Se species. The three studied matrices showed similar pH, but varying ionic strength, T and Eh (Table 5.2).

Table 5.2 Physicochemical character and constituents of the three matrices.

Sample	pH	Eh	*I*	T	O_2	Ca	Fe	K	Mg	Mn	Na	Cl^-	NO_3^-	SO_4^{2-}
			mol L^{-1}	°C	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}
Groundwater	7.26	-38	0.008	4.3	2.88	60.2	2.3	10.3	3.5	n.d	91.4	147.9	3.4	47.5
River water	7.05	-26	0.005	8.8	9.04	47.8	0.3	4.5	4.4	0.2	34.1	48.0	6.9	38.9
Lake water	7.35	-43	0.016	8.3	6.13	98.5	n.d	3.1	14.1	n.d	129.7	265.0	n.d	149.9

I: ionic strength n.d.: not detected

5.3.2 Stability of As(III) and As(V)

The initial concentrations of As(III) and As(V) were 18.0 µg L^{-1} of As(III) and 11.0 µg L^{-1} of As(V) in groundwater, around 5.0 µg L^{-1} of As(III) and As(V) in both, lake water and river water. Considering that 5.0 µg L^{-1} of As(III) and As(V) were spiked to all subsamples after sampling. The concentration of As(III) and As(V) in original groundwater was around 13.0 and 6.0 µg L^{-1}. In lake and river water, however, hardly any As was detected. In general, As(III) and As(V) were both preserved successfully in those samples prepared with EDTA combined with acid (HCl, HNO_3, formic acid or acetic acid) over a period of 11 weeks (Fig. 5.2). For the samples without any preservation (Gw/o, Lw/o and Rw/o), both As(III) and As(V) were lost. In particular As(V) immediately disappeared, while As(III) dropped significantly at first but only disappeared completely in week three. This indicated that both As(III) and As(V) were strongly adsorbed by Fe-(oxy) hydroxide and/or Mn-(oxy)hydroxide, and the adsorption occurred within an hour. However the adsorption of As(V) seemed even faster than As(III). In contrast, the samples preserved with EDTA alone (GE, LE and RE) showed remarkable behavior. In lake water (LE) and river water (RE) almost no changes

of As(III) and As(V) were observed. But in groundwater (GE) As(III) was partially oxidized to As(V), which was indicated by the slight decrease of As(III) and the corresponding increase of As(V) up to the third week of the experiment (Fig. 5.2).

Apparently, EDTA alone was ineffective to preserve the distribution of As(III) and As(V). Samanta and Clifford (2006) reported similar results noting that EDTA alone failed to preserve As species in synthetic water samples without light. Numerous studies showed that in the presence of Fe the preservation of As species became more complicated, because oxidation and adsorption would normally occur. In groundwater with neutral pH, Fe(II) can be oxidized by oxygen and thus facilitate As(III) oxidation (Hug et al., 2001; Zhao et al., 2011). Oxidation of Fe(II) also leads to the precipitation of Fe(III)(hydr)oxides. However, once Fe(III)(hydr)oxides are formed, the oxidation rate of As(III) becomes slow (Hug et al., 2001), and As(V) and As(III) (to a lesser extent than As(V)) are adsorbed onto Fe(III)(hydr)oxides. That was in agreement with our results that in EDTA alone preserved samples where Fe and Mn were chelated by EDTA, oxidation of As(III) was observed (in groundwater). On the other hand, in non-preserved samples where Fe-(oxy) hydroxide and/or Mn-(oxy)hydroxide formed, adsorption of As(III) and As(V) was observed. Previous studies showed that As(V) mainly formed inner-sphere complexes on Fe-(oxy)hydroxides and As(III) in some cases existed as a combination of inner-and outer-sphere surface complexes (Goldberg and Johnston, 2001; Ona-Nguema et al., 2005). This may explain why both As(III) and As(V) showed strong adsorption in our study, and As(V) an even stronger adsorption than As(III) (Fig. 5.2).

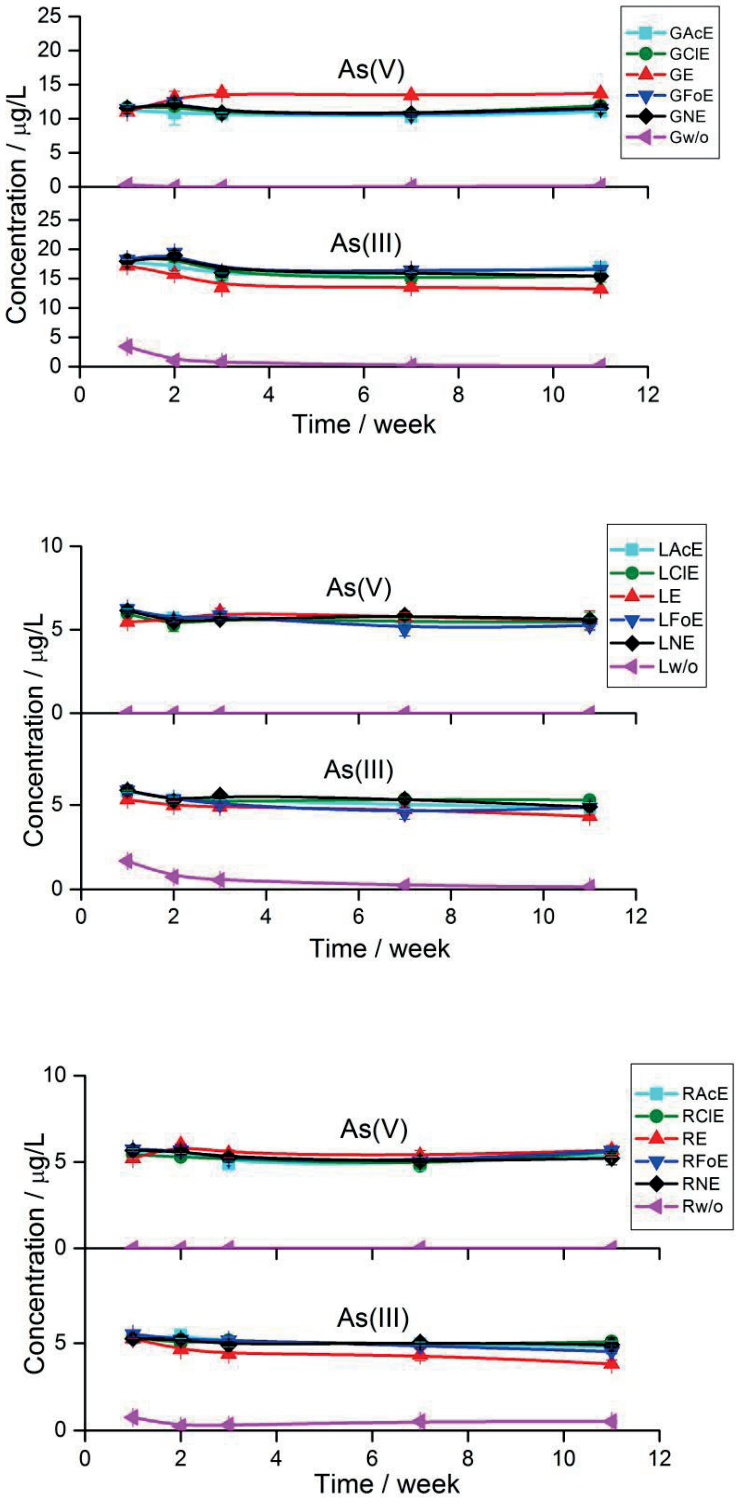

Fig. 5.2 The stability of As(III) and As(V) in groundwater(G), lake water(L) and river water (R). Preserved samples were stored at 4 °C in the dark. Non preserved samples (Gw/o, Lw/o and Rw/o) were stored at room temperature in the presence of light. E-EDTA, Cl-HCl, N-HNO_3, Fo-Formic acid, Ac-Acetic acid, *e.g.* GAcE is groundwater preserved with EDTA and acetic acid; GE is groundwater preserved with EDTA alone; Gw/o is groundwater without preservation.

At pH 3, HCl, HNO_3, formic acid and acetic acid had more or less the same influence on preserving As, Sb and Se species (Figs. 3 to 5). Nevertheless, for longer periods of

storage the combination of an organic acid (formic acid and acetic acid) with EDTA may stabilize the pH of samples better than a combination with a strong inorganic acid (HCl and HNO_3) due to the formation of a buffer system. In our study time scale up to 11 weeks, however, no remarkable differences were observed.

5.3.3 Stability of Sb(III) and Sb(V)

About 5.0 µg L^{-1} of Sb(III) and Sb(V) were added to all subsamples. Again the samples preserved with EDTA combined with acid (HCl, HNO_3, formic acid or acetic acid) successfully preserved Sb(III) and Sb(V) over 11 weeks. In those samples without preservation (Gw/o, Lw/o and Rw/o; with neutral pHs) Sb(III) was missing, indicating adsorption by newly formed Fe-(oxy)hydroxide and/or Mn-(oxy)hydroxide. On the other hand Sb(V) kept constant except in lake water where Sb(V) decreased gradually. Remarkably, EDTA alone failed to preserve Sb(III) and Sb(V) distribution in all matrices, i.e., groundwater, lake water and river water. It can be observed that Sb(III) decreased gradually and disappeared at week three, meanwhile Sb(V) increased accordingly and reached its equilibrium in the third week, indicating that Sb(III) was now completely converted to Sb(V). The first measurement (Fig. 5.3) showed that for those samples preserved with EDTA alone (GE, LE and RE), Sb(III) was detected at a value lower than spiked (5.0 µg L^{-1}), while Sb(V) was detected higher than spiked. In addition, in non-preserved samples, especially lake and river samples, Sb(V) was also detected higher than those preserved with an EDTA acid combination. The results indicated that oxidation of Sb(III) to Sb(V) occurred before measurement. Besides, the big drop of Sb(III) indicated strong adsorption by Fe-(oxy)hydroxide and/or Mn-(oxy)hydroxide. It is worth noting that for all the samples Sb(III) was detected around 2.0 µg L^{-1} higher than spike, about 7.0 µg L^{-1} in general. However, the gained Sb(III) was unlikely from reduction of Sb(V), because Sb(V) remained constant during the whole storage. In fact, remarkable difference was observed between chromatograms of preserved samples (with EDTA, 5.0 µg L^{-1} Sb(III)) and the standard (with de-ionized water, 5.0 µg L^{-1} Sb(III)). The former chromatogram showed a larger and higher peak for Sb(III) than latter. This indicated that in standards the chelation of Sb(III) with EDTA (from the mobile phase) was incomplete, thus Sb(III) was partially retained in the column, which led to a smaller and broader peak. As a result, the slope of the calibraton curve was lower than expected, a phenomenon observed previously (Kolbe et al., 2012), which leads to the suggestion of addition of EDTA to both, samples and standards before measurement.

Since all samples were filtered through a 0.45 μm membrane after sampling and the preserved samples were stored at 4 ^{o}C in the dark, microbial activity and photochemical reactions were minimized. Besides, the dissolved O_2 related oxidation is generally sluggish although oxygen is a strong oxidant from a thermodynamic point of view (Leuz, 2006). Thus the oxidation of Sb(III) in non-preserved samples and EDTA alone preserved samples (Fig. 5.3) was most likely caused by Fe(III). Belzile et al. (2001) observed a fast and complete oxidation of Sb(III) to Sb(V) by Fe-(oxy) hydroxide after a few days following pseudo-first order rate laws. The potential mechanisms involved are: (i) adsorption of Sb(III) and formation of surface complex with Fe(III)-oxyhydroxide (one Sb(III) on two Fe(III) sites); (ii) transfer of two electrons from Sb to two Fe atoms; (iii) release of oxidized Sb(V) and reduced Fe(II). The reason why EDTA alone failed to preserve Sb and As species, while EDTA at lower pH showed a successful preservation for up to 11 weeks might be due to the pH increase caused by EDTA addition. Higher pH accelerated the oxidation of Fe^{2+} and resulted in a higher concentration of dissolved Fe(III) which potentially was capable to oxidize Sb(III) or As(III) (Gault et al., 2005). On the other hand, at lower pH (pH = 3) the oxidation of Sb(III) or As(III) was hampered due to the complete chelation of Fe^{3+} with EDTA. In comparison, As(III) was oxidized much less than Sb(III).

Sb species have more complexing propertes than As and Se. First of all, Sb(V) existed in various forms in solution due to its inability to chelate with EDTA. A previous study (Wu and Pichler, 2014) showed that at a pH above 4.5, Sb(V) existed in the form of the dominant species of $Sb(OH)_6^-$ and even Sb-polymer (if any). In the present work, the behavior of Sb(V) was further investigated at a pH below 3. In this pH range Sb(V) dominantly is present as $Sb(OH)_6^-$ and trace amounts of $Sb(OH)_5$ due to hydrolysis (Tella and Pokrovski, 2012), which is shown by the chromatograms of the three samples (Fig. 5.5). As for Sb(III), the complexation of Sb(III) with EDTA was widely studied. Bhat and Iyer (1965) found that the normal complex species of SbY^- (Y = EDTA) exists in the pH range from 1.8 to 3, with the stability constants of log K_{SbY}^{SbO} = 24.80. The complex converted to free ions of $Sb(OH)_2^+$ (SbO^+) at pH < 1.5. Hydroxy complexes are formed above the pH of 4 ($Sb(OH)Y^{2-}$ in the pH range from 4 to 5.5 and $SbY(OH)_2^{3-}$ in the pH range from 5.7 to 7, with the stability constants of 8.69, and 7.8 respectively.

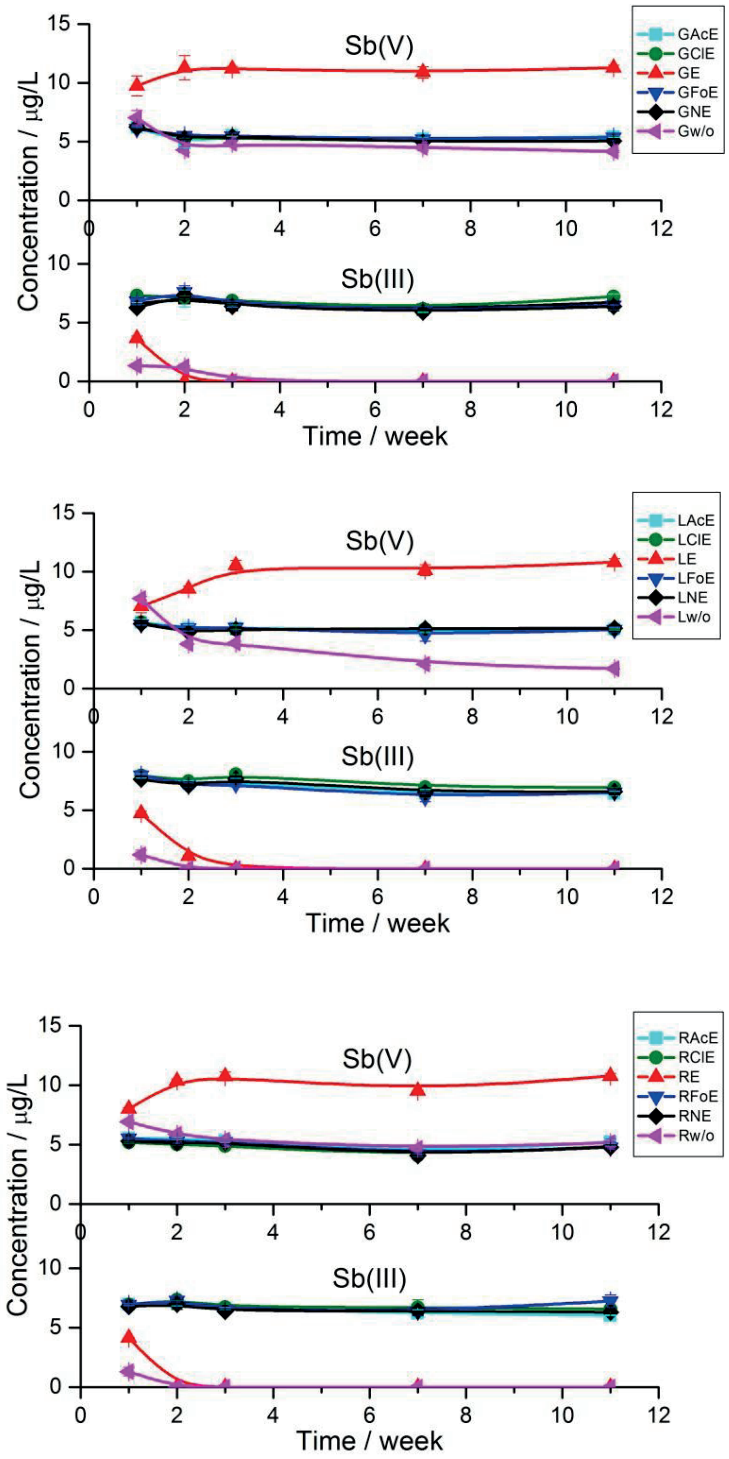

Fig. 5.3 The stability of Sb(III) and Sb(V) in groundwater(G), lake water(L) and river water (R). Preserved samples were stored at 4 °C in the dark. Non preserved samples (Gw/o, Lw/o and Rw/o) were stored at room temperature in the presence of light. E-EDTA, Cl-HCl, N-HNO_3, Fo-Formic acid, Ac-Acetic acid, *e.g.* GAcE is groundwater preserved with EDTA and acetic acid; GE is groundwater preserved with EDTA alone; Gw/o is groundwater without preservation.

Therefore, pH was adjusted to around 3 when using EDTA as preservative for various water samples due to the high stabiliy constant it forms. Besides, Fe(III) started forming Fe-(oxy)hydroxide due to hydrolysis when pH was higher than 3. Previous studies reported that Sb(III) was adsorbed on the surface of Fe(III)hydroxides by inner-sphere

surface complexation at pH 7.7, and that it has a higher affinity for the solid phase than Sb(V) (Mitsunobu et al., 2010). In addition, the solubility of Sb(III) species is much lower than that of Sb(V) species because Sb(III) forms neutral (or no anionic) hydroxide species of $Sb(OH)_3^0$ in this pH range. In agreement in our study Sb(III) was missing in non-preserved samples (Gw/o, Lw/o and Rw/o), while Sb(V) remained stable (except in lake water) (Fig. 5.3).

Compared to As(V), Sb(V) occupies a wider redox range (from Eh = 360 to -140 mV, pH 8), suggesting that Sb(III) could be oxidized at more negative Eh than As (Mitsunobu et al., 2006). On the other hand, the strong adsorption by most solid phases and the low solubility of Sb(III) at a neutral pH is a viable explanation why Sb(V) is the dominant species in most natural environments. Both Sb(III) and As(III) form inner-sphere surface complexes on the goethite surface, thus having similar adsorption behavior. But Sb(III) is a stronger Lewis base than As(III) and the surface sites can be considered as Lewis acids, which explains the stronger binding of Sb(III) than As(III). The adsorption of Sb(V) is more complicated, which is amongst other factors related to pH, ionic strength and initial Sb(V) concentration (Leuz et al., 2006).

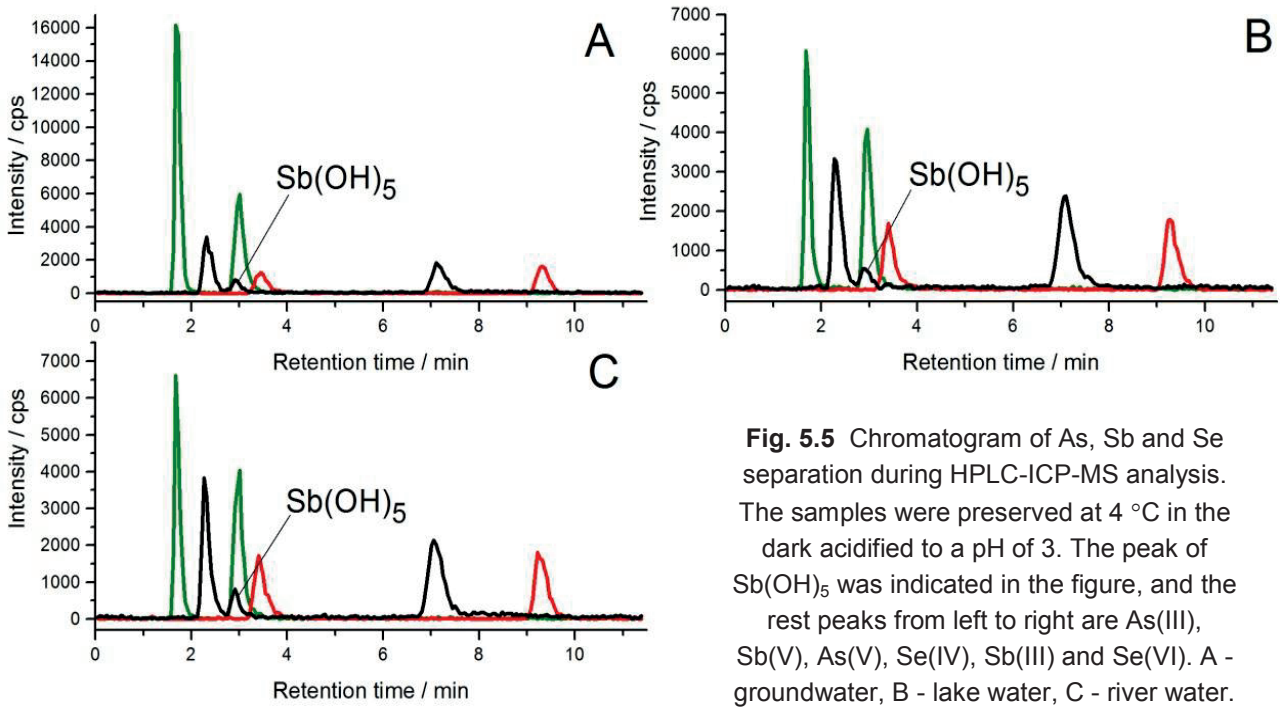

Fig. 5.5 Chromatogram of As, Sb and Se separation during HPLC-ICP-MS analysis. The samples were preserved at 4 °C in the dark acidified to a pH of 3. The peak of $Sb(OH)_5$ was indicated in the figure, and the rest peaks from left to right are As(III), Sb(V), As(V), Se(IV), Sb(III) and Se(VI). A - groundwater, B - lake water, C - river water.

5.3.4 Stability of Se(IV) and Se(VI)

15.0 µg L^{-1} Se(IV) and Se(VI) were added to the subsamples of groundwater, river water and lake. In those samples without preservation (Gw/o, Rw/o and Lw/o), Se(VI) concentration remained unchanged during the duration of the experiment (11 weeks), while Se(IV) was already missing at the first measurement. That indicated that Se(IV) was adsorbed onto Fe-(oxy)hydroxide and/or Mn-(oxy)hydroxide strongly within a short time period of hours and that no oxidation of Se(IV) to Se(VI) occurred during the time. In contrast to observations for As and Sb, EDTA alone was able to preserve the distribution of Se(IV) and Se(VI) in the groundwater, river water and lake water matrices. The EDTA combination with acid (HCl, HNO_3, formic acid or acetic acid) showed the same results. Reddy et al. (1995) investigated the effects of redox potential on the stability of Se(IV) and Se(VI) in groundwater and considered the more stable species to be Se(VI). They did not observe reduction of Se(VI) to Se(IV) over the Eh range from 444 to -280 mV. Since oxidation of Se(IV) to Se(VI) was not observed , the mobility of Se(IV) seemed more susceptible to adsorption. In our experiment Se(IV) was lost immediately in non-preserved samples due to adsorption (Fig. 5.4), however, from the third week a gradual increase of Se(IV) was observed. Considering that the concentration of Se(VI) remained constant, the increase must have been caused by desorption of Se(IV), probably due to the competitive adsorption with the increased OH^- (Mandal et al., 2009). Because an increase of pH (from around 6.2 to around 7.1) was observed during the duration of experiment for the non-preserved samples. EXAFS studies of Se(IV) and Se(VI) adsorption on Fe-(oxy)hydroxides suggested that Se(IV) was adsorbed as an inner sphere surface complex, while Se(VI) was adsorbed mainly as an outer sphere hydrated complex (electrostatic interaction between Se(VI) ions and surface charge) (Rovira et al., 2008; Duc et al., 2003) or a mixture of outer and inner sphere surface complexes on hydrous ferric oxide (HFO) (Peak and Sparks, 2002). Thus the adsorption of Se(VI) is strongly affected by pH and ionic strength, while Se(IV) is not influenced by ionic strength (Duc et al., 2003).

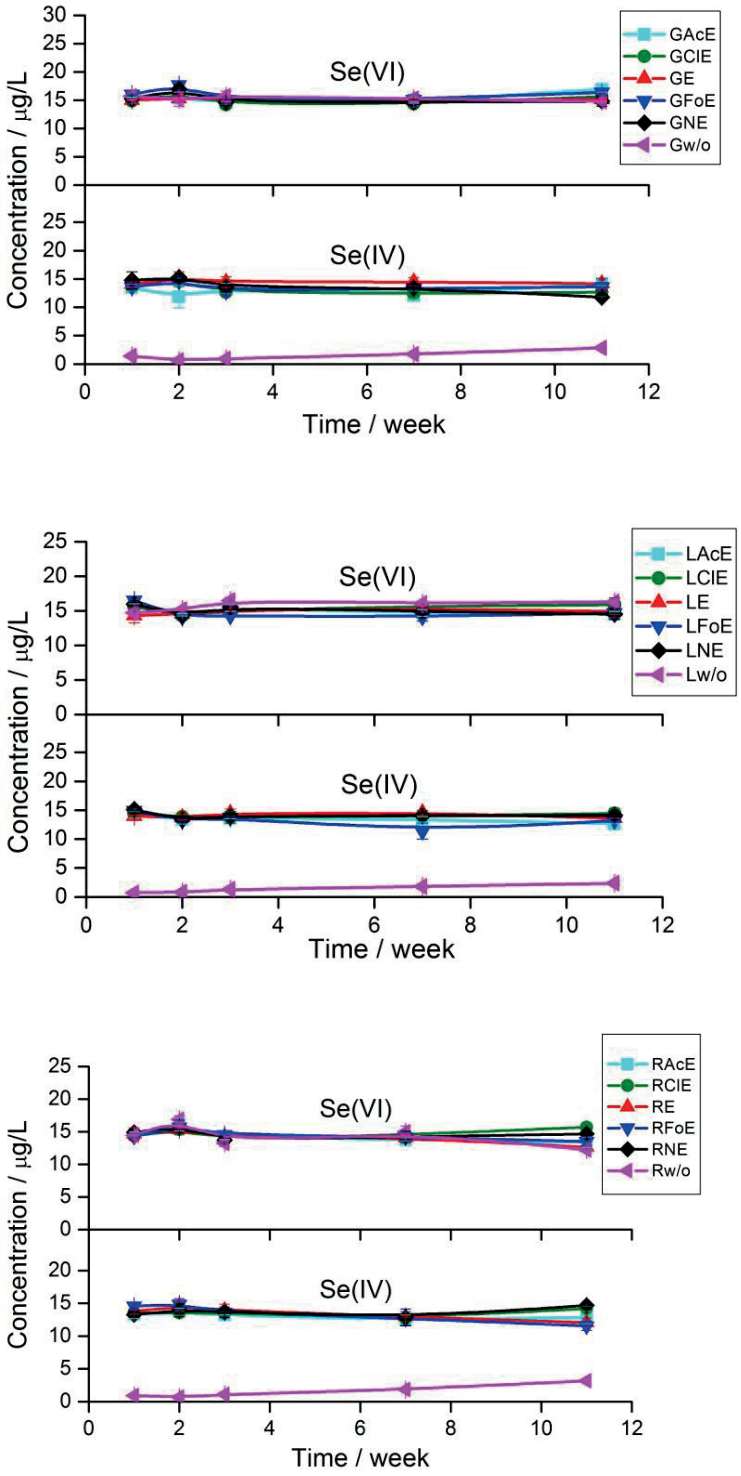

Fig. 5.4 The stability of Se(IV) and Se(VI) in groundwater(G), lake water(L) and river water (R). Preserved samples were stored at 4 °C in the dark. Non preserved samples (Gw/o, Lw/o and Rw/o) were stored at room temperature in the presence of light. E-EDTA, Cl-HCl, N-HNO_3, Fo-Formic acid, Ac-Acetic acid, *e.g.* GAcE is groundwater preserved with EDTA and acetic acid; GE is groundwater preserved with EDTA alone; Gw/o is groundwater without preservation.

5.4 Summary and Conclusions

In this work preservation strategies for As, Sb and Se redox couples in Fe- and Mn-rich water samples was tested. Matrices of groundwater, lake water and river water were studied. At a sample pH of 3 and in the dark (absence of light) EDTA successfully preserved the six desired species for up to 11 weeks at 4 °C. To adjust the pH to 3, various acids, such as HCl, HNO_3, formic acid and acetic acid were used and tested.

1) The metalloids As, Sb and Se are all redox sensitive elements with relatively high mobility in natural waters, nevertheless they behave differently during preservation and storage with respect to stability, redox behavior, chromatographic complexing and adsorption on Fe-(oxy)hydroxide or/and Mn-(oxy)hydroxide.

2) EDTA alone could preserve Se species distribution in all studied matrices, but failed to preserve As and Sb. The combination of EDTA with an acid (HCl, HNO_3, acetic acid or formic acid) to adjust sample pH to 3 successfully preserved the studied redox couples in groundwater, river water and lake water.

3) The chromatography of Sb revealed that the abundance of Sb(V) varied with pH. Sb(V) was present mainly as $Sb(OH)_6^-$ and minor $Sb(OH)_5$ at a pH of 3.

4) Oxidation affected the redox couples to different degrees. Under the same conditions Sb(III) was completely oxidized to Sb(V), while As(III) was partially oxidized and Se(IV) was not oxidized at all.

5) Lower-valence state species (As(III), Sb(III) and Se(IV)) were easily be adsorbed on Fe-(oxy)hydroxide and/or Mn-(oxy)hydroxide, indicating strong adsorption affinity. Besides, As(V) also showed a strong adsorption by Fe-(oxy)hydroxide and/or Mn-(oxy)hydroxide. That may be explained by the fact that they mainly form inner sphere complexes. Higher-valence state species such as Sb(V) and Se(VI), however, were not adsorbed in most cases. Because they may form outer sphere complexes and were bonded via weak electrostatic adsorption.

Acknowledgements

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6. As and Sb redox species in hydrothermal waters from Bali and Java, Indonesia

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Abstract

In the present work, the previously reported method for simultaneous speciation of As, Sb and Se redox couples by SF-ICP-MS coupled to HPLC was applied to analyze As(III, V) and Sb(III, V) in hot spring samples from Java and Bali Island, Indonesia. The result showed that samples from Bali were mainly HCO_3-rich, samples from Java mainly Cl-rich. As concentrations (As(III) + As(V)) on Java were generally higher (up to 9220.83 µg L^{-1}) than in samples from Bali (with the highest concentration of less than 40 µg L^{-1}). In five of the analyzed samples (B1, B2, B3, B7 and J1), an unidentified As species was detected, in two (B7 and J1) the unidentified species was even the dominant species. This indicates that other processes such as seawater feeding were likely involved during distribution of As species. Sb concentrations were generally much lower than As, with the highest concentration of 61.4 µg L^{-1}. The analyzed samples were classified as Cl-type, SO_4-type and HCO_3-type according to their relative concentration. The relationship between As, Sb and Cl, B was analyzed, As showing a better correlation to Cl and B than Sb. Generally, in HCO_3-type hydrothermal waters As(V) seemed undoubtedly the dominant species. With Cl- and SO_4-type samples, it was more complicated. Since extremely high concentration of Cl might be originated from either magma degassing (HCl gas) or seawater input, other oxidation processes may be involved during distribution of As species. In Cl-type hydrothermal waters As seemed initially discharged in form of As(III) from host rock-water interaction. If hydrothermal waters underwent no further dilution by groundwater (J2) or was diluted by groundwater but without presence of oxidizing agents such as Fe and Mn (J7 and J8), As(III) remained the dominant species. However, when hydrothermal waters were further affected by seawater feeding (B7, J1, J5 and J6), As(V) was dominant. In SO_4-type hydrothermal waters, either As(III) or As(V) could be the dominant species, but meanwhile the other species may also exist substantially. As for Sb species, Sb(V) was generally the dominant species in the analyzed samples, probably due to concentrations of Sb being at trace level and very easily oxidized or adsorbed (co-precipitated).

6.1 Introduction

As and Sb are ubiquitously present in hydrothermal fluids, *e.g.* volcanic hot springs (Wilson et al., 2012; Stauffer and Thompson, 1984; Stefánsson and Arnórsson, 2005) and hydrothermal fluids emerging at the seafloor near mid-ocean ridges (MOR) or in back-arc basins (BAB) and island arcs (IA) (Breuer and Pichler, 2013). Up to date, the understanding of As and Sb geochemistry and the distribution of their species is still limited. Compared to normal natural environments, elevated concentrations of As and Sb are often observed in hydrothermal fluids. Previous studies found that in geothermal waters As and Sb are highly mobile depending on chemical water type, salinity and temperature (Arnórsson, 2003) and can be good indicators for the extent of rock leaching and water-rock reaction. Besides, the ratios of trace elements, particularly As, to conservative elements such as Cl and B have been used to characterize geothermal systems (Stauffer and Thompson, 1984) or quantify contributions in geothermal environments (Mroczek, 2005).

The existing forms of As and Sb vary under different conditions. Mambote et al. (2003) made an investigation into ferric-arsenic(III)-sulfate system using thermodynamic modeling. As(V) is the main species in oxic conditions and As(V) can be distributed as soluble species, such as AsO_2^-, AsO_4^{3-}, H_3AsO_4, $H_2AsO_4^-$ and $HAsO_4^{2-}$. The dominant species is strongly dependent on pH and ligands present in the solution. In hydrothermal fluids from shallow-water island arcs, $As(OH)_3$ is predominant at a pH ranging from 5 to 7 and reducing conditions ($E_h < 0$), whereas at higher temperatures and pressure as in deep-water from MOR and BAB, $H_2AsO_4^-$ occurs more often (Breuer and Pichler, 2013). Since a hydrothermal system includes aqueous solution and volatile vapor, it has been proved that both phases are capable of transporting As, and in vapor phase $As(OH)_3$ (aq) being the dominant species (Pokrovski et al., 2002). However, for hot springs with temperatures below 300 °C, the amount of As transported by vapor phase can be ignored. It has been shown that in hot springs with temperatures less than 95 °C, the main dissolved species are $As(OH)_3$ and $As(OH)_4^-$ or $H_2AsO_4^-$ and $HAsO_4^{2-}$. While chloride-complexes or $HAsS_2$-AsS_2 levels are negligible even in presence of rich chloride or H_2S (Criaud and Fouillac, 1989). Sb usually existed as Sb(V) in solution under oxic conditions. SbO_2^+ is the species existing under very acidic conditions and $Sb(OH)_6^-$ or and $Sb(OH)_5$ were the main species present under wide acidic, neutral and alkaline conditions (Pitman et al., 1957; Takayanagi and Cossa, 1997; Tella and Pokrovski,

2012). Besides, antimonic acid tends to condense to polymers or chelate with organic ligands as pH increases (Gate and Richardson, 1961a,b). For Sb(III), $Sb(OH)_2^+$ (very acidic condition) and neutral $Sb(OH)_3$ (pH range from 2 to 10) were the main species in acidic and alkaline conditions (Pitman et al., 1957). The XAFS study showed that in NaCl-HCl solutions, typical for acidic high-temperature hydrothermal fluids, Sb(III) existed as $Sb(OH)_3$ and Sb-OH-Cl. Sb species can also partition into vapor phase during vapor-brine separation under acidic conditions in magmatic-hydrothermal systems. However, this process remained minor in neutral low to moderate-temperature solutions (≤ 250-300 °C). Recently, the occurrence of antimony-polysulfide species in geothermal waters has been proved (Planer-Friedrich and Scheinost, 2011; Mosselmans et al., 2000), which could play an important part in Sb transportation in hydrothermal systems.

Compared to common natural environments, As and Sb showed unique behavior in hydrothermal systems. Many things have an effect on As and Sb concentrations in hydrothermal fluids, *e.g.* physicochemical, including temperature (controlling phase separation of the fluids and leaching process in the host rock), pressure, pH, As and Sb species mobility and speciation, adsorption and desorption. Sb has been determined as being closely associated to other elements such as B, Mo, W, S, Au and U (Stefánsson and Arnórsson, 2005; Boyle and Jonasson, 1984). Besides, Sb can also act as a potential geochemical tracer of water-rock interaction and fluid sources, due to the large isotope variations of hydrothermal sulfide deposits and surface environments or magmatic rocks (Rouxel et al., 2003). Arsenic is presumed showing similar chemical behavior as Sb, because both have 3+ and 5+ oxidation states and can form oxyanions in solution. A highly positive correlation between As and Sb concentrations has been found in hydrothermal waters (Sakamoto et al., 1988). Arsenic also plays an important part in the formation of hydrothermal ore deposits. In general, the chemistry of hydrothermal systems has been intensively studied. But, for As and Sb speciation in hydrothermal fluids, only scarce reports can be found (Criaud and Fouillac, 1989; Yokoyama et al., 1993; Wilkie and Hering, 1998). A reason might be the inability of preserving As and Sb species identically as in original matrices during storage. In addition, the lack of a simple simultaneous speciation method for As and Sb also retarded the study of As and Sb species in hydrothermal systems. Traditional methods include two basic analysis runs of duplicate samples, one for total concentration and one for one of the species. The other species thus can be calculated by subtraction of the

two. However, discrepancies were often observed between total concentration and the sum of different species. Because other species may also exist in substantial amount, such as organic species or As(or Sb)-sulfur species. These could also be dominant species in certain environments (this work, sample B7 and J1).

Based on the method we have developed for simultaneous speciation analysis of As, Sb and Se redox couples by SF-ICP-MS coupled to HPLC (Wu and Pichler, 2014). In the present work we analyzed As and Sb redox couples in hot spring samples from Bali and Java Island, Indonesia. Distribution and existing forms were studied as well as the association of As and Sb with HCO_3^{-}, SO_4^{2-} and Cl^- and B.

6.2 Materials and methods

6.2.1 Instruments

A sector field ICP-MS (SF-ICP-MS) (Element 2, Thermo) was used for As(III, V) and Sb(III, V) detection. A Conikal nebulizer and Scott type spray chamber were used as injection system. The outlet of the HPLC column was connected via PEEK capillary tubing to nebulizer. For As and Sb, isotopes of ^{75}As and ^{121}Sb were monitored. The potential $^{40}Ar^{35}Cl^+$ has been confirmed not an interference on As analysis, as Cl^- was eluted at a different time from As(III) and As(V) (Wu and Pichler, 2014). The element 2 was connected to an Accela 1250 Pump (Thermo) which was equipped with an auto sampler. A Hamilton PRP-X100 (Hamilton, Reno, USA) anion-exchange column (250 mm × 4.1 mm, 10 µm) protected with a guard column was used. The detection and chromatography conditions are listed in Table 6.1. The pH of all solutions was determined using a pH-meter (pH 340, WTW).

6.2.2 Reagents, standards and certified reference materials

All solutions were prepared with double deionized water obtained from a Millipore water purification system (MilliQ Advantage A10, 18 MΩ cm).

Stock solutions (1000 mg L^{-1} for each species) were prepared as follows: As(III) from As(III) oxide (As_2O_3, Sigma-Aldrich) dissolved in 4 g L^{-1} NaOH (Merck). As(V) from sodium arsenate dibasic heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$, Sigma-Aldrich) dissolved in water. Sb(III) from potassium antimonyl tartrate trihydrate ($C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$, Sigma-

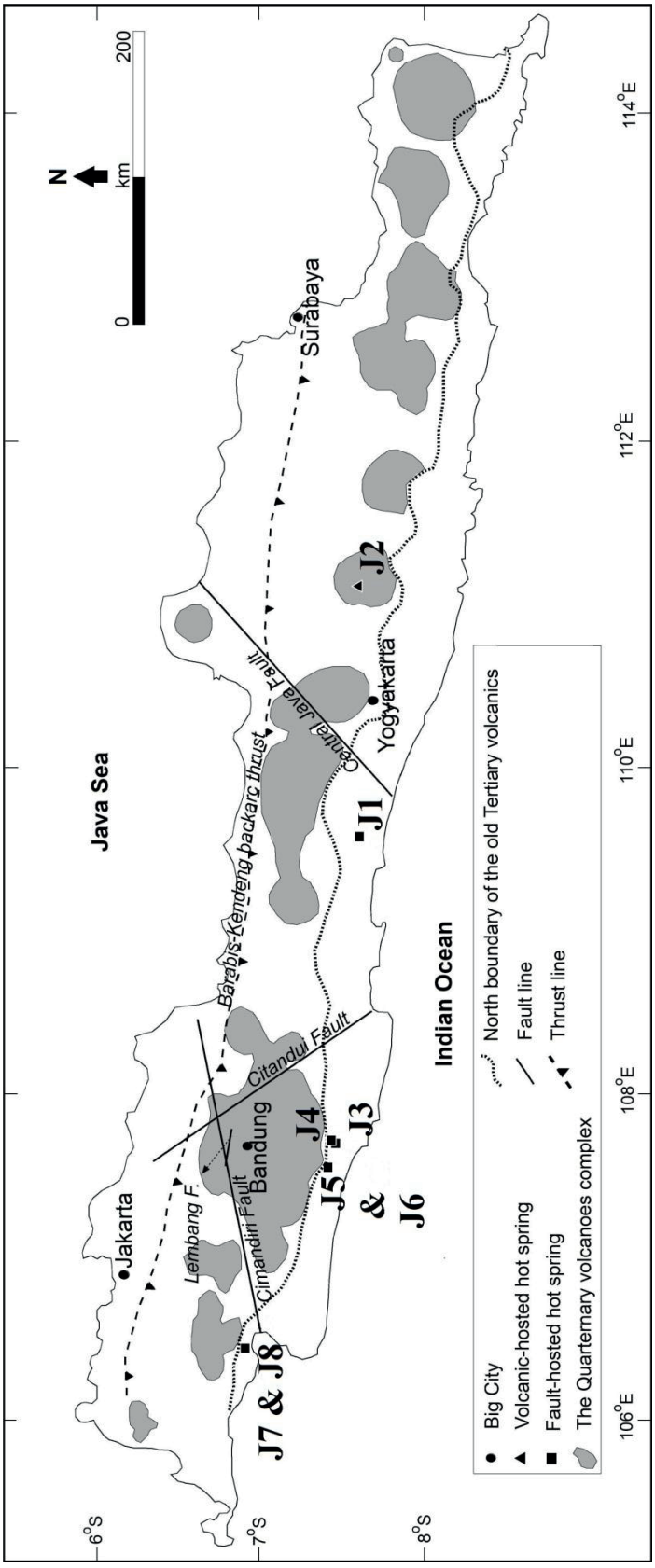
Aldrich) dissolved in water. Sb(V) from potassium hexahydroxoantimonate (H_6KO_6Sb, Fluka) dissolved in water. Se(IV) from sodium selenite (Na_2O_3Se, Sigma) dissolved in water. Se(VI) from sodium selenate (Na_2O_4Se, Sigma-Aldrich) dissolved in water. All stock solutions were kept at 4 °C in the dark and standards and mix standards of lower concentrations were prepared daily by appropriate dilution with water.

The mobile phase was prepared using EDTA (p.a. AppliChem), the pH was adjusted with ammonium (Suprapur, Merck) and formic acid (98-100%, Merck). The mobile phase was filtered through a 0.45 µM membrane (Whatman) before use. Methanol (for HPLC, ≥ 99.9%, Sigma-Aldrich) was used in combination with EDTA solution as mobile phase.

The following certified reference materials were used for quality control: SRM 1643e (NIST, National Institute of Standards and Technology) and CRM-SW (High-purity Standards). SRM 1643e contains 60.45 ± 0.72 µg L^{-1} As and 58.30 ± 0.61 µg L^{-1} Sb. CRM-SW contains 20.00 µg L^{-1} As.

Table 6.1 The detection and chromatographic conditions used during analysis.

Nebulizer	Conikal nebulizer
Spray chamber	Scott type spray chamber
Monitored isotopes	^{75}As, ^{121}Sb
Resolution mode	High resolution (HR) or Medium resolution (MR)
Column	PRP-X100 (250 mm × 4.1 mm, 10 µm) (Hamilton, Reno, USA)
Mobile phase	30 mM EDTA (97%) + methanol (3%)
pH	4.7 (adjusted with formic acid)
Flow rate	1.5 mL min^{-1}
Injection volume	50 µL
Species	As (III, V), Sb (III, V)


N
0
200
km
Java Sea
Indian Ocean
Jakarta
Bandung
Surabaya
Yogyakarta
Lembang F.
Cimandiri Fault
Baribis-Kendeng backarc thrust
Central Java Fault
Citandui Fault
J1
J2
J3
J4
J5
&
J6
J7 & J8
Big City
Volcanic-hosted hot spring
Fault-hosted hot spring
The Quarternary volcanoes complex
North boundary of the old Tertiary volcanics
Fault line
Thrust line
6°S
7°S
8°S
106°E
108°E
110°E
112°E
114°E

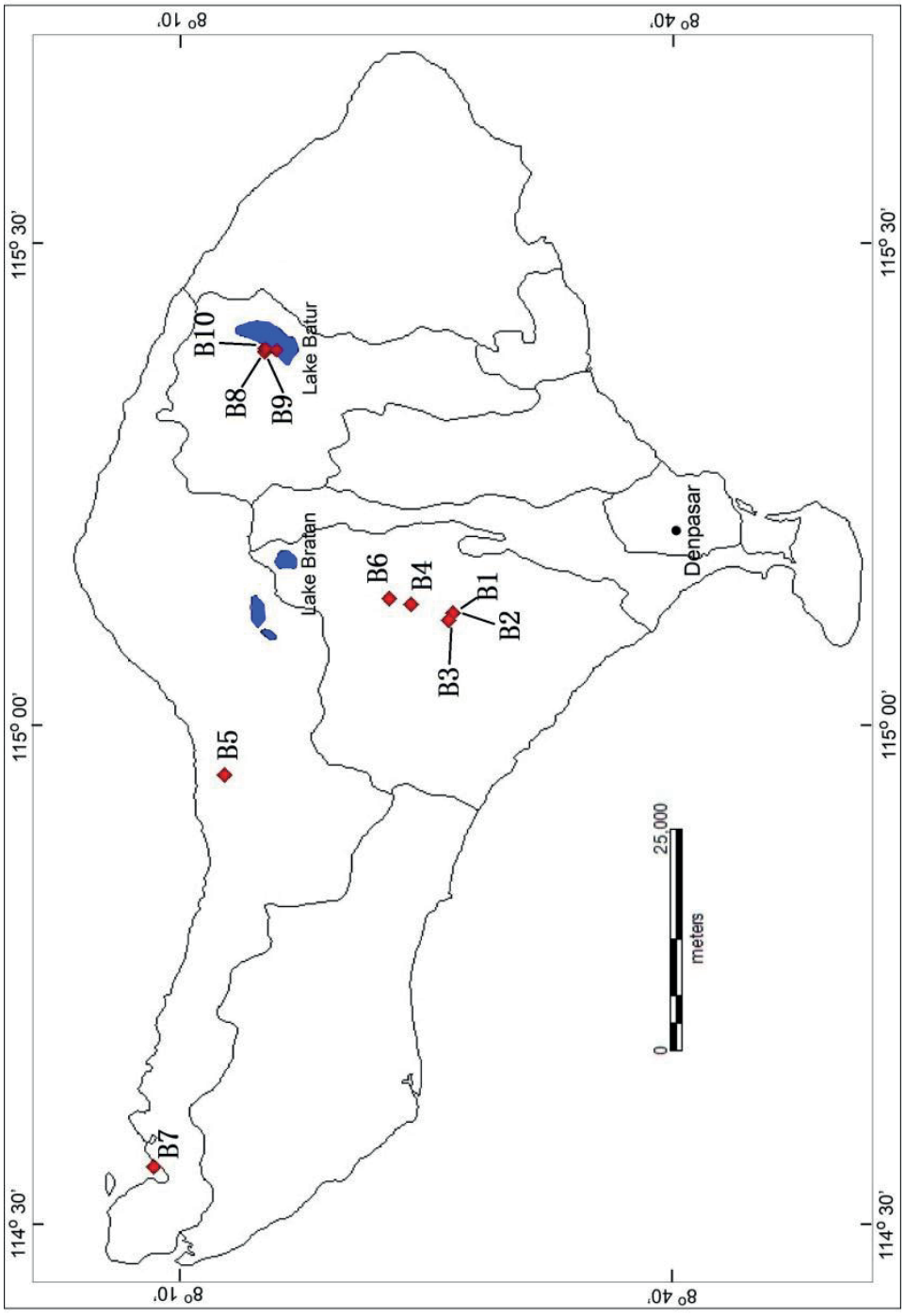


Fig. 6.1 Sampling locations on Java and Bali Island, Indonesia.

6.2.3 Geological setting, Sampling and Preservation

Java, an island in the Indonesian archipelago, is part of a long volcanic arc that extends from Sumatera to Nusa Tenggara. The older rocks (Tertiary) are andesitic, while the younger (Quarternary) rocks are more alkaline (Soeria-Atmadja et al., 1994). The volcanoes and faults on Java are host to at least 62 geothermal fields (Setijadji, 2010), most of which are located in the Quaternary volcanic arc, including 7 developed geothermal fields, i.e. Dieng, Darajat, Kamojang,Wayang-Windu, Gunung Salak, Patuha and Karaha-Bodas. Bali Island is part of the Sunda-Banda volcanic islands arc, extends for approximately 4700 km, from Sumatera Island in the west to Damar Island in the east (Hamilton, 1979). The surface of the island is covered dominantly by volcanic rocks resulted from multiple volcanisms. Jembrana volcanic dominates the western part of the island, Buyat-Bratan and Batur volcanic products in the middle, while Agung and Seraya volcanic products in the east. Underlying these volcanic products are the Tertiary sedimentary rocks and limestone, which only expose in relatively small areas in the eastern, south and western parts of the island (Hadiwidjojo et al., 1998).

In this work hot spring samples were collected from representative hot springs scattered over Bali and Java Island (Fig. 6.1). Some samples (B8, B9 and B10) were situated close to crater lakes, some (B7 and J1) close to seawater. The physicochemical parameters such as pH and temperature were measured in the field (Table 6.2). All samples were filtered through a 0.45 µm membrane immediately after sampling. Three splits of samples were prepared; one for anion measurement, one for cation measurement (acidified with 1% concentrated HNO_3) and one for speciation (acidified with HNO_3 and preserved without light). All samples were stored in polyethylene bottles and transported to lab for further study.

Table 6.2 Sampling locations, geotype (volcano-hosted or fault-hosted) of hot springs, and physicochemical parameters and constituents of the analyzed samples. Some data were from (Purnomo and Pichler, 2014)

Sample	GeoType	Location	Temp	pH	Ca	Cl	HCO_3	SO_4	Si	Mn	Fe	B	Na
			°C		mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}
B1	V	Bali	38.8	6.5	122.4	377.0	1466.4	0	76.0	0.0	0.0	8.0	263.4
B2	V	Bali	38.8	6.62	122.5	363.7	1525	0	76.0	0.0	0.0	7.6	270.2
B3	V	Bali	42.6	6.42	135.1	443.9	1555.5	0	80.7	0.0	0.0	9.1	309.3
B4	V	Bali	41.8	6.53	211.5	61.2	2235.0	111.6	72.7	0.7	0.0	4.2	234.4
B5	F	Bali	37.2	6.19	68.4	17.2	773.4	2.2	73.1	0.0	1.3	1.9	109.2
B6	V	Bali	45.2	6.12	54.3	16.6	634.4	165.9	97.2	0.1	0.7	5.4	123.0
B7	F	Bali	44.6	7.75	51.3	902.1	31.7	200.2	11.6	0.0	0.0	1.1	526.8
B8	V	Bali	39.9	7.45	46.0	159.3	463.6	370.3	56.5	0.0	0.0	1.9	294.2
B9	V	Bali	43.1	7.28	46.6	136.2	458.7	325.2	61.9	0.0	0.0	1.9	277.8
B10	V	Bali	40.6	7.41	47.4	146.9	488	328.6	57.2	0.0	0.0	2.0	281.6
J1	F	Kebumen	38.9	8.22	2082.9	8671.8	31.2	21.6	0.0	0.0	0.0	14.5	2436.3
J2	V	Lawu	38.4	6.1	510.7	5948.7	835.7	256.3	42.9	0.5	9.3	93.2	2979.0
J3	F	Pakenjeng	59.9	7.4	225.5	126.0	40.2	940.1	27.1	0.0	0.0	7.2	224.3
J4	F	Pakenjeng	43.1	7.5	215.6	131.7	42.7	960.0	26.5	0.03	0.0	7.6	256.9
J5	F	Cilayu	70.3	8.1	68.3	1387.2	372.1	408.1	79.3	0.08	0.1	58.2	1101.5
J6	F	Cilayu	45.1	7.9	227.0	3210.5	289.1	156.6	82.7	0.2	0.1	47.5	1797.4
J7	F	Cisolok	102.0	8.1	41.2	305.6	129.3	235.5	66.3	0.02	0.0	3.5	285.7
J8	F	Cisolok	100.0	8.0	52.3	276.9	161.0	222.7	58.8	0.06	0.0	3.2	257.5

V: volcano-hosted; F: fault-hosted

6.3 Results

6.3.1 Chemical compositions

The temperature for most of the analyzed samples ranged from 37 to 70 °C, except for J7 (102.0 °C) and J8 (100.0 °C), indicating that the vapor phase is not significantly important for transporting As or Sb species. As for pH, all samples had neutral pH, ranging from 6.0 to 8.5. Geological types of the sampling locations were classified into volcano-hosted and fault-hosted, based on their position in either a volcanic complex or fault zone (Purnomo and Pichler, 2014). Basically, volcano-hosted geothermal systems had higher HCO_3^- than fault-hosted geothermal systems, due to degassing and subsequent CO_2-water reaction in volcano-hosted systems. The water types of samples, indicated as HCO_3^-, Cl^- and SO_4^{2-} in Table 6.3, were classified according to Cl-SO_4-HCO_3 ternary diagram (Chang, 1984; Giggenbach, 1991; Nicholson, 1993). Based on their position in the diagram, the water could be divided into neutral chloride, acid sulfate and bicarbonate which can be indicators of hydrothermal origins. *E.g.* neutral Cl-SO_4 water could be: 1) a mixture of alkali chloride water and acid sulfate water; 2) resulted from oxidation of H_2S to SO_4^{2-} in alkali-chloride water or dissolution of S from rock followed by oxidation (Ellis and Mahon, 1977); acid-SO_4 water arises from oxidation of H_2S to SO_4 near the surface and most of its composition is dissolved from surface rock (Ellis and Mahon, 1977); HCO_3^- water, as mentioned above, might be resulted from degassing and CO_2 - water reaction. Neutral Cl-Na water was originated from host rock-water interaction. Additionally, elements of B, Si, Mn and Fe were measured, as they were potentially important geo-indicators or could play an important role in adsorption and oxidation of As and Sb species. It can be seen that the concentrations of Fe and Mn in the analyzed samples were generally at a very low level (0 - 0.7 mg L^{-1} for Mn, and 0 - 9.4 mg L^{-1} for Fe), indicating a less important role in oxidizing As and Sb species. The B concentration ranged from 1.1 to 93.2 mg L^{-1}.

B and Cl ratios have been extensively used to identify volcanic and hydrothermal processes, such as rock-water interaction, magmatic origin and seawater influence (Arnórsson and Andrésdóttir, 1995; Purnomo and Pichler, 2014; Valentino and Stanzione, 2003). From Fig. 6.2 it can be seen that B and Cl were in good linear relationship for all analyzed samples except for sample B7 and J1. B7 and J1 were excluded, possibly being influenced by seawater, thus causing an altered B/Cl ratio. The

lower Cl/B ratio of sample J5 might be explained by B redistribution during andesitic host-rock leaching (Purnomo and Pichler, 2014), as Cl has been reported more conservative than B. This indicated that B and Cl in those hydrothermal waters originated from the same sources, and were not obviously influenced by secondary phenomena such as seawater mixing, rock-water interaction.

Table 6.3 Concentration of As and Sb species for samples from Bali (B) and Java (J). Two CRMs were used for mass balance assessment. Water types were classified according to the relative concentration of bicarbonate, chloride and sulfate.

	Sample	As(III) $\mu g\ L^{-1}$	As(V) $\mu g\ L^{-1}$	Sb(III) $\mu g\ L^{-1}$	Sb(V) $\mu g\ L^{-1}$	Type
Bali	B1	0.3	1.9	< DL	0.3	HCO_3
	B2	0.3	1.4	< DL	0.2	HCO_3
	B3	0.3	2.0	< DL	0.2	HCO_3
	B4	< DL	20.2	0.3	0.3	HCO_3
	B5	< DL	0.5	< DL	< DL	HCO_3
	B6	0.9	36.9	0.3	0.5	HCO_3
	B7	0.3	0.4	0.3	0.2	Cl
	B8	< DL	13.8	< DL	0.2	HCO_3
	B9	< DL	17.0	< DL	0.2	HCO_3
	B10	< DL	15.0	< DL	0.3	HCO_3
Java	J1	< DL	0.3	< DL	0.2	Cl
	J2	7310.3	1910.6	< DL	< DL	Cl
	J3	797.6	144.4	< DL	0.6	SO_4
	J4	46.3	636.2	< DL	1.0	SO_4
	J5	178.4	3056.5	< DL	61.4	Cl
	J6	10.5	2615.4	< DL	16.6	Cl
	J7	100.1	1.7	1.0	4.8	Cl
	J8	88.1	1.7	2.8	0.4	Cl
CRM	SRM 1643e	0.7	57.8	< DL	59.9	-
	Certified(SRM 1643e)	60.5(As in total)		58.3(Sb in total)		-
	CRM-SW	< DL	20.1	< DL	< DL	-
	Certified(CRM-SW)	20.0(As in total)				-

DL: detection limit

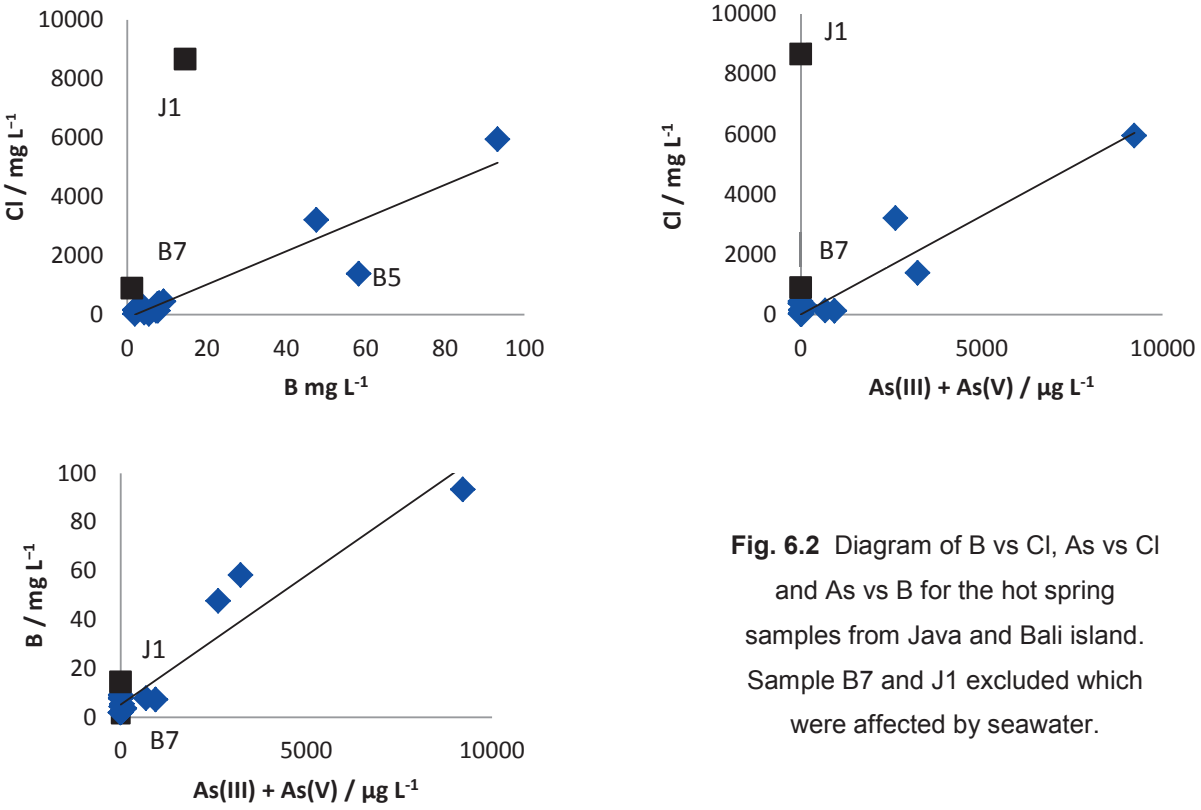


Fig. 6.2 Diagram of B vs Cl, As vs Cl and As vs B for the hot spring samples from Java and Bali island. Sample B7 and J1 excluded which were affected by seawater.

6.3.2 As(III) and As(V) in hot spring water

Two CRMs: SRM 1643e (NIST, National Institute of Standards and Technology) and CRM-SW (High-purity Standards) were analyzed for mass balance assessment. In SRM 1643e, 57.8 µg L^{-1} As(V) and 0.7 µg L^{-1} As(III) were detected. Total As (60.5 µg L^{-1}) was calculated combining As(III) and As(V) species. As for Sb only Sb(V) (59.9 µg L^{-1}) was detected. For CRM-SW, only As(V) (20.1 µg L^{-1}) was detected. For both the CRMs total concentration of As and Sb were in good agreement with the certified value (Table 6.3).

It can be seen from Table 6.3 that for samples from Bali Island, the concentrations of As were at trace level, ranging from 0.5 µg L^{-1} (B5) to 37.7 µg L^{-1} (B6). While for samples from Java Island, As concentrations were generally detected very high (up to 9220.8 µg L^{-1} was detected in J2), except for J1 (only 0.3 µg L^{-1} was detected). Additionally, the concentrations of As varied in a large range for samples from Java, from 89.8 µg L^{-1} (J8)

to 9220.8 μg L^{-1} (J2). As species (As(III) and As(V)) were also variable in different samples. Apparently, in samples J2, J3, J7 and J8, As(III) was the dominant existing form, though in samples J2 and J3 a relative amount of As(V) was detected as well (1910.6 μg L^{-1} and 144.4 μg L^{-1} respectively). In other samples As(V) was more abundant than As(III). From Table 6.3 and Fig. 6.5, it can be seen that samples B1, B2, B3, B4, B5, B6, B8, B9 and B10 were HCO_3-type. Samples B7, J1, J2, J5, J6, J7 and J8 were Cl-type, and, J3 and J4 were SO_4-type. In HCO_3-type samples As mainly existed in form of As(V). In Cl-type samples, it was more complex; three samples (J2, J7 and J8) were obviously As(III) dominated, and two samples (J1, J5 and J6) were As(V) dominated. In Cl-type sample B7, however, both As(III) and As(V) were detected in relative amount. In SO_4-type samples (J3 and J4), one is As(III) dominant (J3) and the other As(V) dominant (J4), meanwhile the other kind of species was also detected significantly (144.38 μg L^{-1} As(V) for J3 and 46.31 μg L^{-1} As(III) for J4).

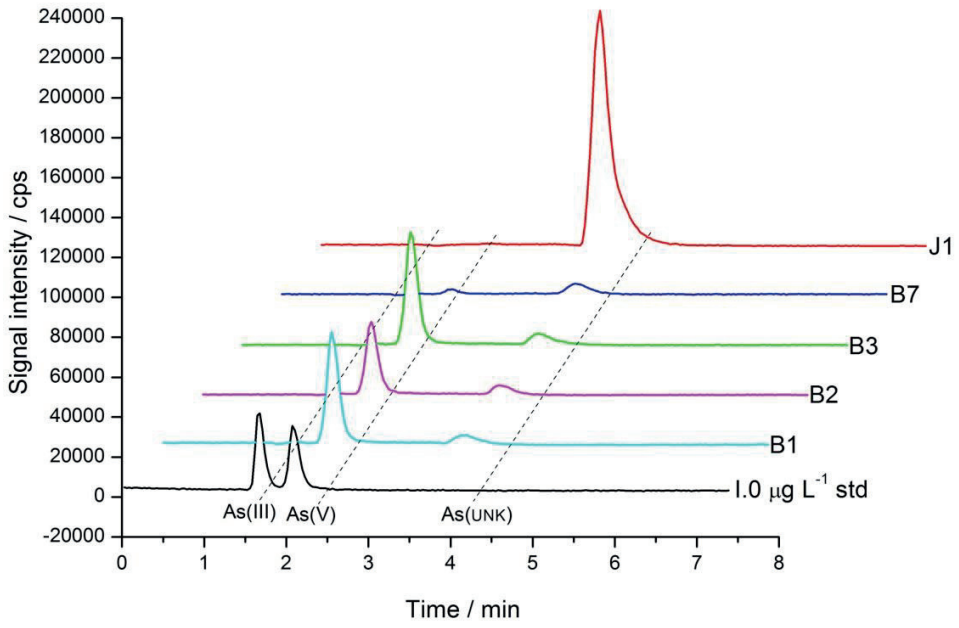


Fig. 6.3 The chromatogram of As species for sample B1, B2, B3, B7, J1 and 1.0 μg L^{-1} standard. The retention times were: 1.68 min for As(III), 2.10 min for As(V) and 3.60 min for As-unknown species.

From the chromatogram of samples B1, B2, B3, B7 and J1 (Fig. 6.3), an unknown peak at around 3.6 min was observed. In samples B1, B2 and B3, the unidentified species

was at trace level. In sample B7 and J1, however, this unidentified species was the dominant species, particularly in sample J1 (Fig. 6.3). Considering that As(III) and As(V) inorganic species eluted out from chromatogram at 1.68 min and 2.11 min during measurement (compared to standard containing 1.0 μg L^{-1} As(III) and As(V) plotted in Fig. 6.3 as well), the "unknown peak" should be As organic species. The explanation is that these two hydrothermal waters were influenced by seawater, which was indicated by extremely high Cl concentrations and high Cl/B ratios (Table 6.2).

The correlation of As and Sb species to SO_4^{2-}, Cl^- and HCO_3^- had been extensively studied. Criaud and Fouillac (1989) studied the distribution of As(III) and As(V) in geothermal waters from France, Dominica, New Mexico, the USA and Bulgaria. The results indicated that As(III) was the main species in hydrothermal systems, the distribution of As(III) and As(V) may vary a lot under different conditions. *E.g.* bicarbonate waters are generally enriched in oxidized As, whereas acid sulfate springs have a variable As(III). However, other factors may also play an important part. Firstly, the kinetics of oxidation and reduction are in dynamic balance in hydrothermal systems. Secondly, secondary phenomena such as mixing, cooling and water-rock interaction would affect the distribution of As(III) and As(V). Besides, during rising of hydrothermal fluids to the surface, As distribution could also be modified by biogeochemical processes. Another study of thermal springs of Yellowstone National Park showed that in representative acid-sulfate-chloride thermal springs As(III) was the dominant species from sources, and rapid oxidation of As(III) to As(V) was observed downstream after discharge via chemical or biological processes (Wilkie and Hering, 1998; Langner et al., 2001). Arsenic was found occurring more or less exclusively as the trivalent arsenite species in shallow-water hot springs in Tutmu Bay (Price and Pichler, 2005; Pichler et al., 1999).

In our study the relationship between As(As(III)+As(V)) and Cl was studied for all samples from Bali and Java. The diagram of As vs Cl (Fig. 6.2) showed that As concentration was closely associated to Cl in the analyzed samples except for samples B7 and J1 (as has been discussed, they may have been influenced by sea water), indicating that they may have a common source. Positive correlations between As and Cl in hydrothermal systems were also found by other researchers, and had been used as a tool to predict the origin of As, such as magmatic sources or water-rock leaching (Stauffer and Thompson, 1984). In another study Cl was used as inert tracer to evaluate

the contribution of As from natural outflows of geothermal fields and discharge of geothermal effluent to the river (Mroczek, 2005).

In addition, the correlation of As and B was investigated, as B is known acting conservatively once in solution, it can function as a tracer as well. Fig. 6.2 shows that a certain correlation between As and B was obtained for the samples from Java and Bali. The correlations of As vs Cl, As vs B and B vs Cl indicated that these elements behaved similarly and probably had common sources. Previous studies have shown that As in geothermal waters was possibly originated from rock leaching rather than of direct magmatic origin (Ellis and Mahon, 1964, 1967; Ewers, 1977). Based on this, host rock-water interaction was the possible origin for these conservative elements in the analyzed samples.

6.3.3 Sb(III) and Sb(V) in hot spring water

Compared to As, the data for Sb was much sparser. The concentrations of Sb in the studied hydrothermal waters were generally much lower than As, and Sb species were more easily affected by oxidation and adsorption (or co-precipitation). Besides, Sb has complexing properties, and can form complexations with organic compounds. Up to date many speciation methods for Sb have been developed, and hot spring waters were used to validate methods. But scarce reports are found with respect to Sb speciation as a tool for a better understanding of hydrothermal systems. Measurement was problematic, because of low concentrations of Sb species present in hydrothermal waters. In addition, preservation of Sb species was a big challenge for researchers especially for samples with high concentrations of oxidizing agents, such as Fe and Mn.

Table 6.3 shows that concentrations of Sb were generally at trace level for samples collected from both Bali Island and Java Island, the highest concentration being 61.4 μg L^{-1} (sample J5, from Java). Sb(V) was detectable for most of the samples, while Sb(III) was generally below detection limit (B1, B2, B3, B5, B8, B9, B10, J1, J2, J3, J4, J5 and J6). Generally, Sb(V) was the main existing form. In samples B4 (HCO_3-type), B6 (HCO_3-type) and B7 (Cl-type) both Sb(III) and Sb(V) were detected at relative amounts. In sample J8 (Cl-type), however, Sb(III) was apparently the dominant species: 2.76 μg L^{-1} Sb(III) compared to 0.38 μg L^{-1} Sb(V). In sample J7 (Cl-type) Sb(V) was obviously the dominant species, though 0.94 μg L^{-1} Sb(III) was detected. In samples B5 and J2,

neither Sb(III) nor Sb(V) was detected. The results showed that the distribution of Sb species seemed less correlated with Cl^- or HCO_3^- than As. On the other hand, Sb species have been verified as being closely correlated to sulfide in hydrothermal fluids due to the formation of Sb-sulfide complexes (Planer-Friedrich and Scheinost, 2011; Sherman et al., 2000; Mosselmans et al., 2000). The mineralization of Sb in geothermal systems at depth has been studied. Compared to the conservative behavior of Sb in low sulfide, natural environment, Sb mobility in hydrothermal systems was largely controlled by the presence of sulfide and changes in pH and temperature. Another report on the behavior of Sb released from surface geothermal features in New Zealand showed that the concentration of Sb showed distinct diurnal variations in sulfide-rich feature (Wilson et al., 2012). The sulfide-sulfate equilibria and direct stibnite oxidation may play a role in this process. While in another location in the absence of rich sulfide, Sb exhibits little diurnal fluctuation. As for Sb species distribution, previous study showed that the oxidized form of Sb(V) was the dominant species in surficial environments, however Sb(III) can also be present of significant amounts especially in volcanic and magmatic hydrothermal fluids (Zotov et al., 2003). This was in agreement with our results.

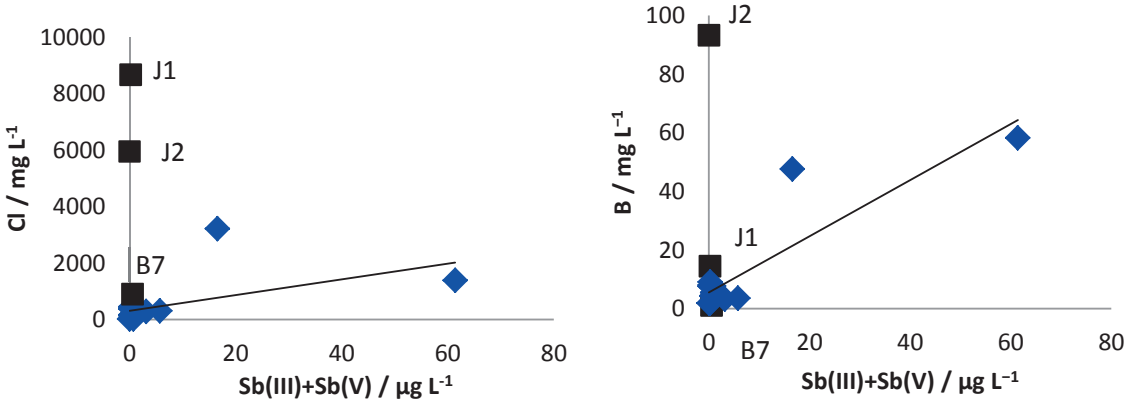


Fig. 6.4 Diagram of Sb vs Cl and Sb vs B for the analyzed samples. Sample B7, J2 and J1 were not included. Sample B7 and J1 were affected by seawater and J2 was detected with the highest Fe concentration of 9.4 mg L^{-1}.

Fig. 6.4 showed that correlation of Sb to Cl and B. It can be seen that both B and Cl were less correlated to Sb(III) + Sb(V) than to As, even samples B7, J1 and J2 were excluded. Noteworthy, sample J2 was largely deviated from linear (Sb under detection limit), probably due to adsorption by Fe-(oxy)hydroxide, as J2 was the only sample that contained a Fe concentration high up to 9.38 mg L^{-1}. The results indicated that Sb was more variable than As.

6.4 Discussion

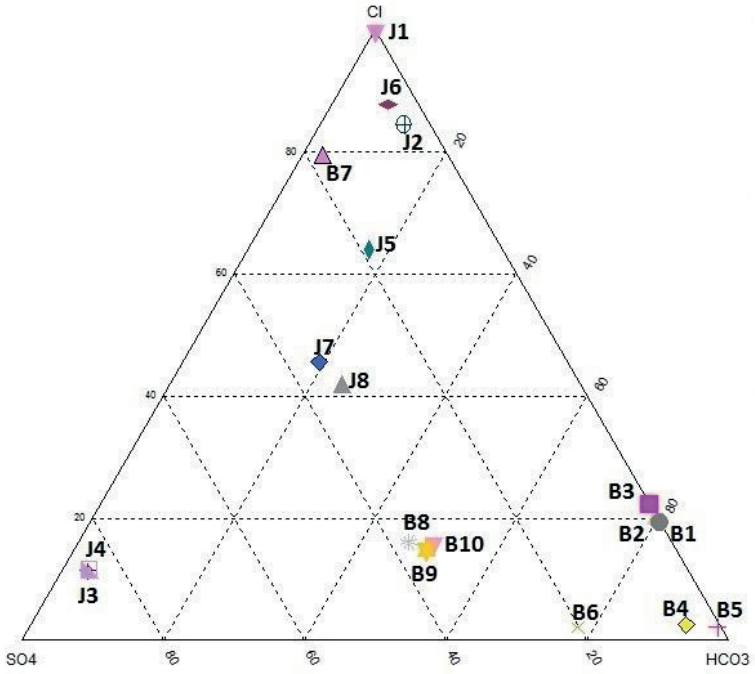


Fig. 6.5 SO_4-HCO_3-Cl ternary diagram of hot spring samples from Bali and Java. Some data were from Purnomo and Pichler, (2014)

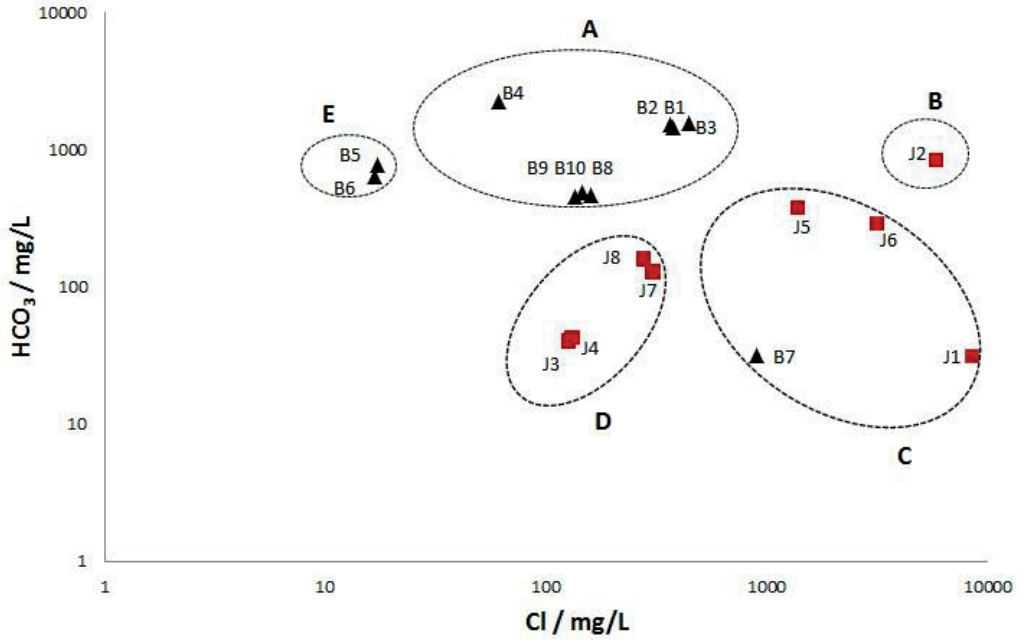


Fig. 6.6 HCO_3 vs. Cl diagram for the analyzed samples. A and B formed in the margin of "primary neutralization zone", but B is closer; C were diluted by seawater; D and E were diluted by groundwater. Some data were from Purnomo and Pichler, (2014)

Fig. 6.5 showed the SO_4-HCO_3-Cl ternary diagram of hot spring samples from Bali and Java. J3 and J4 were obviously SO_4-type. B7 was the only sample from Bali that was categorized as Cl-type thermal water. Samples J7 and J8, located in the middle of the diagram, had a moderate concentration of Cl^-, SO_4^{2-} and HCO_3^-. In HCO_3-type hydrothermal waters, oxidized As(V) seemed undoubtedly the dominant species. Although after discharge from sources the distribution of As(III) and As(V) may be modified by biogeochemical processes during the rise of the fluid to the surface. As for SO_4-rich geothermal water, arsenic species and total As are variable. This type of geothermal waters most likely derived from oxidation of S (from host rock leaching) or H_2S (from magma degassing). Considering the two sulfate type samples from Java (J3 and J4) being fault-hosted and the pH being neutral, the enrichment of sulfate could be caused by host rock leaching of S followed by oxidation. During this process As(III) would also be partially or even mostly oxidized. This explains why in these two samples As(V) was dominant but As(III) also occurred in relative amount. In Cl-rich geothermal waters, the distribution of As(III) and As(V) could be influenced by Cl origins, *e.g.* seawater feeding. Normally extremely high concentrations of Cl were resulted from either magma degassing of HCl(g) or seawater feeding. It can be seen from table 6.2

that both J1 and J2 contained extremely high concentrations of Cl^- (8671.8 mg L^{-1} for J1 and 5948.7 mg L^{-1} for J2). However, J1 is fault hosted (the concentrations of HCO_3^- and SO_4^{2-} are very low, 31.2 mg L^{-1} and 21.6 mg L^{-1} respectively, compared to 835.7 mg L^{-1} and 256.4 mg L^{-1} in sample J2) and influenced by seawater with massive Ca (2082.9 mg L^{-1}). Besides, the dominance of unknown organic As species indicated that microbial activity was involved. This explained the fact that almost no inorganic As species (only 0.3 µg L^{-1} As(V)) could be detected, due to the formation of organic species and secondary phenomenon, *e.g.* adsorption or co-precipitation by minerals. Sample J2 however, is volcano-hosted and formed in the margin of the "primary neutralization" zone (Giggenbach, 1988), and was still not further diluted by groundwater. In the other two fault-hosted samples J5 and J6, the extremely high concentrations of Cl^- (Cl^- concentration of 1387.2 and 3210.5 mg L^{-1} respectively) indicated that they were influenced by seawater input as well (Purnomo and Pichler, 2014), thus other oxidizing processes may be included and in these two samples As(V) was dominant. In fault-hosted samples J7 and J8, the moderate concentrations of Cl^-, HCO_3^- and As indicated that they may be diluted by groundwater to the same extent after discharge from host rock and water reaction. However, As(III) remained dominant, probably due to the absence of other oxidizing agents, such as Fe and Mn (only trace amounts were detected). The influences of seawater and groundwater on hot springs were illustrated in Fig. 6.6 where the samples were grouped in A, B, C, D and E. Group A and B were volcano-hosted, and formed in the margin of "primary neutralization zone", thus had higher concentrations of Cl^- and HCO_3^-. However, group B was closer to the "primary neutralization zone" where Cl^- and HCO_3^- were still rich and still not further diluted. Group C and D were fault-hosted; C was diluted by seawater, and D was diluted by groundwater. E group was volcano-hosted and diluted by ground water.

Sample J2, as has been discussed, may have been affected by adsorption of iron oxides. That may explain the extremely high concentration of As (9220.8 µg L^{-1}) but no presence of Sb. In order to further check the correlation between As and Sb (positive correlation between Sb and As was found by other researchers in geothermal waters (Sakamoto et al., 1988)), the ratios of As(III)/As(V) and Sb(III)/Sb(V) were studied (Fig. 5.7). It can be seen that in most of the samples (B1, B2, B3,B5, B8, B9, B10, J1,J4, J5 and J6) both As(III)/As(V) and Sb(III)/Sb(V) were around 0, indicating that the oxidized species (As(V) and Sb(V)) were the main existing forms. In samples B7 and J8, where

relative amounts of As(III) were detected, Sb(III) was also detected as main existing form. On the other hand, however, big differences were found in samples B4, B6, J2, J3 and J7. In samples B4 and B6, relative amounts of Sb(III) were detected, however for As, As(V) was the dominant species. In samples J2, J3 and J7, where As(III) was much higher than As(V), Sb(V) on the contrary was the dominant species. The results indicated that though As and Sb were both redox sensitive elements and had some similar geochemical behavior in hydrothermal water, their redox species may vary a lot depending on different environments. *E.g.* samples J7 and J8 had similar physicochemical parameters and constituents (Table 6.2) and As(III) was dominant in both samples. However, Sb species showed big difference; in J7 Sb(V) was dominant and in J8 Sb(III) was dominant. This indicated a different process controlling As and Sb species distribution, *e.g.* thermodynamic equilibrium or microbial activity. Besides, Sb was generally much lower than As in concentration in hydrothermal water, and much easier affected by oxidation and adsorption (or precipitation). Sb species can also form complexations with ligands (sulfides, chlorides or other organic ligands) present in hydrothermal solutions. All these factors make Sb more variable in hydrothermal systems (Obolensky et al., 2007; Sherman et al., 2000; Mosselmans et al., 2000).

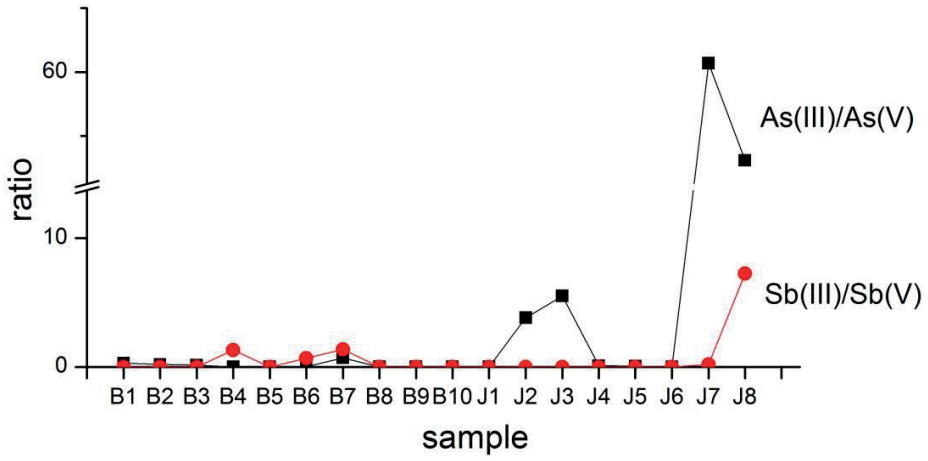


Fig. 5.7 The ratio of As(III)/As(V) and Sb(III)/Sb(V) of the samples from Java and Bali.

Fig. 5.8 showed the distribution of As and Sb species in pH-Eh diagram. It can be seen that for both As and Sb under oxidizing conditions (Eh > 0.1 V), oxidized states of As(V) and Sb(V) were the main existing forms in samples B8, B9, B10, J4, J5 and J6. The existing form of As(V) in these samples was $HAsO_4^{2-}$. On the other hand, under reducing conditions (Eh < -0.2 V), reduced states of As(III) and Sb(III) were detected as main forms in samples J8 and B7. However, for the samples situated in moderate reducing and oxidizing conditions, the distribution of As and Sb species was variable, either reduced or oxidized states could be dominant, *e.g.* samples B1, B2, B3, B4, B5 and B6 were in the zone of H_3AsO_3, however the results showed the oxidized state of As(V) being dominant; sample J3 plotted in the zone of $HAsO_4^{2-}$, As(III) on the contrary was the main form. This indicated that the mobility of As or Sb in natural environment was determined by multi processes, thus prediction of As and Sb species distribution using pH-Eh diagram solely is limited.

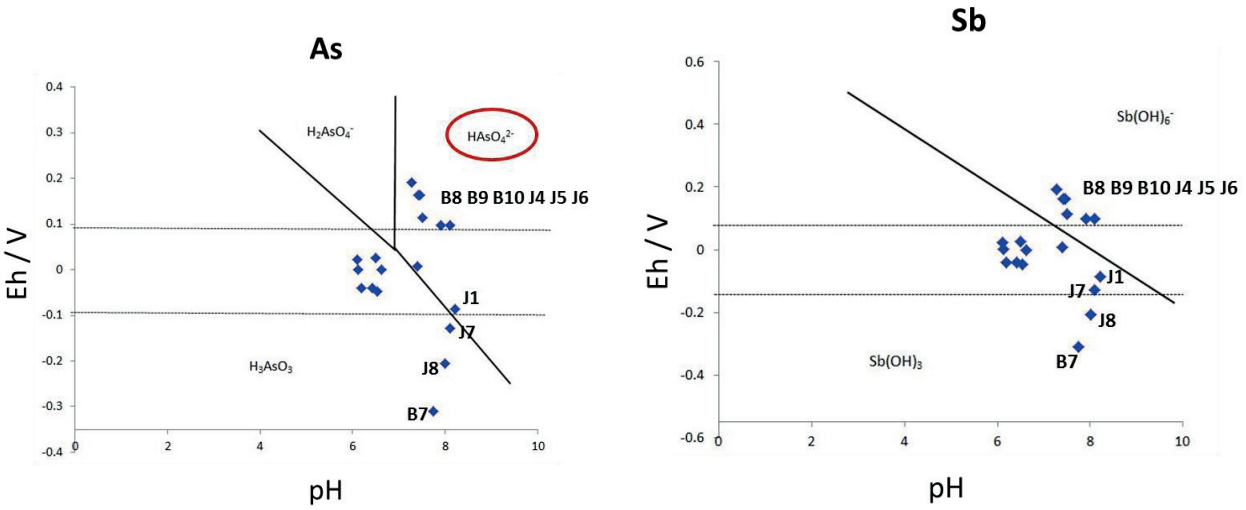


Fig. 5.8 pH vs Eh diagram for the analyzed samples.

6.5 Conclusions

As and Sb inorganic species were analyzed simultaneously for hot spring samples from Java (8 samples) and Bali (10 samples) island, Indonesia. In our work the samples from Java were mainly Cl-type and samples from Bali were mainly HCO_3-type. Arsenic and Sb concentrations varied in a large range and samples from Java were generally much

higher than those from Bali especially for As (up to 9220.8 μg L^{-1}), depending on the type of hydrothermal water or water-rock interaction. In five samples an unidentified species was detected. Two of the samples were most likely affected by seawater, where the unidentified species was even the dominant species. Environmental factors may have strongly influenced the distribution of As and Sb species. Our preliminary speciation results showed that in HCO_3-type hydrothermal waters (mainly volcano-hosted) As(V) was the dominant species, though it was still not clear whether As(V) was the original dominant species or being modified from As(III). In Cl-rich hydrothermal waters, since very high concentration of Cl^- could be originated from either magma degassing (HCl gas) or seawater input, the distribution of As might be influenced by other oxidation processes. When the hydrothermal water was not further diluted by groundwater after discharge or diluted by groundwater but without presence of oxidizing agents such as Fe and Mn, As(III) may remain the dominant species. However, when the hydrothermal waters were further affected by seawater mixing As(V) may be dominant. In SO_4-type hydrothermal waters, As species were also variable, either As(III) or As(V) could be dominant, probably due to different oxidation processes. As for Sb species, Sb(V) was generally the dominant species in the analyzed samples. Compared to As, data on Sb was poorer. The reason may be that concentrations of Sb were at trace level and more mobile than As. In addition, Sb species were much easier affected by oxidation or adsorption (co-precipitation).

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7. Conclusions and perspectives

7.1 Conclusions

Within this thesis, systematic study was carried out from method development for As, Sb and Se redox species to real analysis of hydrothermal samples, from stability study of these species to finding of appropriate preservation strategies. In the first manuscript a simultaneous speciation analysis method focused on inorganic redox species of As(III, V), Sb(III, V), and Se(IV, VI) based on HPLC-SF-ICP-MS was developed and optimized. A thorough validation concerning linearity, retention time stability, detection limits and recovery was made using artificial solution as well as real hot spring samples (from Java, Indonesia). No inter-conversion between species or mass loss during chromatography was observed. In addition matrix influence on species retention time was also checked. The method is characterized with simple eluent composition, short overall analysis time, low detection limit, good linearity and reliable repeatability of retention time, and thus could be safely applied to a variety of fluid samples. In the second manuscript, preservation strategies for As, Sb and Se inorganic species were proposed. Adsorption and redox behavior of these species with the presence of Fe-(oxy)hydroxide and possibly Mn-(oxy)hydroxide were also studied. Some interesting results were obtained, *e.g.* 1) though As, Sb and Se are all redox sensitive metalloids, they behave differently during preservation and storage with respect to stability, redox behavior, chromatographic complexing and adsorption on Fe-(oxy)hydroxide or/and Mn-(oxy)hydroxide; 2) it explained the reason why previously reported EDTA-based preservation method was ineffective, as EDTA can only stabilize Sb(III) under low pH (around 3). Addition of EDTA solely, on the contrary, can even accelerate the oxidation of Sb(III); 3) these redox species showed different adsorption behavior with the presence of Fe-(oxy)hydroxide. As(III), Sb(III), Se(IV)) and As(V) showed a strong adsorption affinity by Fe-(oxy)hydroxide indicating inner sphere complexations. While Sb(V) and Se(VI) were not adsorbed in most cases due to the formation of outer sphere complexes. In the third manuscript, the developed method was applied for the analysis of hydrothermal waters. This work focused on the distribution of As redox species, with comparison to Sb species. The correlation between As (or Sb) species and Cl^- (HCO_3^- or SO_4^{2-}) were studied, as the water type (Cl-, HCO_3- or SO_4-) was good indicator of hydrothermal water sources. Our primary speciation results showed that in HCO_3-rich

thermal waters, As(V) is generally higher than As(III). In SO_4-rich samples, the concentration of As is variable. In addition the influence of seawater feeding on the distribution of As and Sb species was also studied. In seawater-influenced samples, other oxidation processes was most likely involved, because an unknown species were detected as the dominant As species in two samples. For Sb species, Sb(V) was generally the main existing form. Sb concentration (60 μg L^{-1} being the highest) was much lower than As (high up to 9.2 mg L^{-1}) in the analyzed hydrothermal waters from Bali and Java. Samples from Java generally has much higher concentration of As and Sb than those from Bali.

6.2 Perspective

There are still large gaps in our knowledge with respect to the redox behavior of redox sensitive, multi species elements, such as As, Sb and Se in different environment. With the method for simultaneous speciation of As, Sb and Se redox couples, the next step is to:

1) further study the distribution, toxicity and bioavailability in different matrices. Previous studies have shown that all of these are related to oxidation states. However, in some circumstances, thermodynamic predictions are in contradictory to reality. For example, in aqueous environment Sb equilibrium is thought to be controlled by equition: **$Sb(OH)_3 + 3H_2O = Sb(OH)_6^- + 3H^+ + 2e^-$ (Log *K* = -29.8)**. This yields a ratio of Sb(V) to Sb(III) to be $10^{18.4}$ in well aerated oxic water at pH of 6 and Eh of 0.80 V. Thus, Sb(V) should be the dominant species in oxic waters. However, in real natural water systems, both thermodynamically stable species (Sb(V)) and thermodynamically unstable species (Sb(III)) can be detected in relative amount (Takayanagi and Cossa, 1997). Similar results were also observed for Se species.

2) evaluate competitive adsorption of, for example, As and Sb, onto hydrous ferric oxides (HFO) surfaces, which in turn will let us better predict their mobility. Numerous studies have been carried out concerning the adsorption behavior of As(III) and As(V) individually. However, As(III) and As(V) coexist in natural environment, and a recent report showed that there exist a competitive adsorption on HFO surface between As(III) and As(V) in binary system (Qi and Pichler, 2014). To our knowledge, simultaneous adsorption behavior of As and Sb species is still not well understood.

3) investigate the geochemical behavior of As and Sb in hydrothermal systems. The ratio of As(III)/As(V) and Sb(III)/Sb(V) might be a promising tool for advancing comprehension of hydrothermal system.

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